Order Number 9408504

The effects of early experience on the hippocampus

Wilson, Lynn Allison, Ph.D.

The University of Arizona, 1993

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

THE EFFECTS OF EARLY EXPERIENCE ON THE HIPPOCAMPUS

by

Lynn Allison Wilson

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PSYCHOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1 9 9 3

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Lynn Allison Wilson

entitled THE EFFECTS OF EARLY EXPERIENCE ON THE HIPPOCAMPUS

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

Lynn Nadel	1/2/92 Date
A.J. Figueredo	1/2/92 Date
Gary Wenk	1/2/92 Date
	Date
	Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

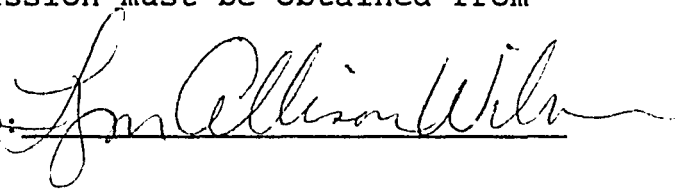
I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director Lynn Nadel — 7/20/93 1/2/92 Date

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under the rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in any part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgement the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: 

ACKNOWLEDGEMENTS

This research has been made possible by the efforts of many people. Lynn Nadel always managed to find the resources necessary to continue with this project. Diane Amend assisted with surgery, and was an invaluable aid in performing the CORT assays. Diane also spent a great deal of time processing the paperwork required for the completion of this project. Stacey Warren spent many hours assisting with the water tank tasks, and performing surgeries. A.J. Figuerado provided statistical assistance, and Gary Wenk generously shared lab facilities and supplies. Some of the funding for this research was provided by a program development grant from the Graduate College of The University of Arizona.

Looking backward to the past, I would like to dedicate this dissertation to the memory of my mother, Sara Frances Booth Pullen.

Looking forward to the future, I'd like to thank my children, Jason, Nikki and Sara for their presence, support and patience.

Table of Contents

List of Illustrations

List of Tables

ABSTRACT

Some experiences occuring early in life affect structure and function of the nervous system. Handling and isolation of infant rats produce physiological and behavioral changes that persist throughout life. These changes may result from interference with the maturation of late developing systems, such as the hippocampus. The research reported here used handling and isolation alone, and in combination, and measured activity, cognitive ability and plasma CORT levels in adult rats. Handling resulted in increased activity, decreased CORT levels, and impaired spatial learning ability. Isolation failed to alter activity levels, impaired spatial ability, and increased CORT levels in females, and reduced them in males. Combining the two manipulations produced no changes in behavior or CORT levels. The results are discussed in terms of altering the manner in which the animals respond to environmental challenges.

The effects of early experience on the hippocampus.

Introduction

Most organisms are remarkably adapted to the environments in which they live. Sensory, perceptual and cognitive capacities of a given species are well suited to the survival needs of its members. Perhaps one of the oldest debates in psychology concerns whether these characteristic abilities unfold according to some genetically predetermined blueprint, or whether they develop in response to stimulation from the environment. For example, in mammals, the ability to perceive the world visually depends on receptor cells in the retina. These cells are genetically determined and provide the ability for a species to perceive some range of sensory input - thus vision would appear to be determined by nature, or simple maturation of the cells responsible for producing it. On the other hand, it has been shown that limiting visual experience during development alters cells in the visual cortex. Kittens exposed only to horizontal lines during early development later fail to respond to lines of other orientations (Mitchell, 1980). Studies such as this demonstrate the effect of experience - or nurture - on structure and function of the nervous system. It has become increasingly clear that both biologically determined maturation and exposure to environmental events play a role

in neural and behavioral development. There are many important questions to be answered in the study of environmental influences on development. These include: What sorts of experience affect development? When in the timing of development does experience exert effects? What physiological and biochemical systems can be altered by experience? Finally, how is the behavior of an organism affected by early experience?

The current study addresses some of these issues. The hippocampus is a structure that, like the visual system, undergoes considerable development after birth. It is of interest to determine whether experience early in life can influence the way that the hippocampus functions later in life. Specifically, two forms of early experience - handling and isolation of rat pups are assessed for their effects on the hippocampus. Two observations form the basis for this work. First, as mentioned above, the hippocampus is a structure that undergoes considerable postnatal maturation, a fact that suggests that experience can influence maturation. Second, handling and isolation are forms of early experience known to alter behavior, and may do so by affecting the development of the hippocampus. Recent experimental evidence suggests that handling of rat pups may alter the portion of the stress response system that is localized in the hippocampus (Meaney, et al, 1988).

Similarly, there is some reason to believe that isolating rat pups from their mother also affects the stress response system (Stanton, Wallstom & Levine, 1989). This study examines the effects of these manipulations on the hippocampal stress response system, and looks at behaviors known to depend on the hippocampus. The goals of this project are to examine ways in which environmental influences may affect hippocampal development, and to explore the idea that hippocampal learning and memory functions are determined, at least to some extent, by the neuroendocrine component of that structure.

Chapter 1: The Hippocampus

The hippocampus was described as early as 1690 (Angevine, 1965), and has generated considerable interest in neuroanatomists, physiologists and psychologists. Even so, there is still disagreement as to its precise functional significance. Perhaps nowhere is the parable of the blind men and the elephant more appropriate than in a discussion of hippocampal function. This structure is involved in learning, memory, emotion and appears to regulate certain forms of behavior. By focusing on any one of these functions, the "elephant" loses some of its complexity and its wholeness. Studies have typically focused on one aspect or another of supposed hippocampal function, and therefore provided evidence for only that particular function.

Extensive damage to the human hippocampus produces a nearly complete loss of ability to form new autobiographical memories (c.f. Milner, 1966). O'Keefe and Nadel (1978) proposed that the hippocampus acts as the neural substrate used to form cognitive maps of space and of the contextual components of experience. Subsequent studies have provided support for this theory: the ability of rats to navigate through large scale space is disrupted by hippocampal lesions (Chozik, 1983; for extension to other species, see also Kesner, 1990). Many experiments that have used nonhuman primates as subjects have demonstrated that the

ability to carry information across a delay in time (delayed nonmatching to sample tasks) is affected by hippocampal lesions (c.f. Aggleton, Blindt & Rawlins, 1989; Zola-Morgan & Squire, 1986).

It is of interest to determine whether these varied deficits have some common functional characteristic. To this end, several theories have recently been put forth. One theory suggests that the hippocampus serves as a directory that "points" to cortical areas in which memories are actually stored (Teyler & DiScenna, 1986). Another proposes the hippocampus as an integrator of cross-modal sensory inputs (Sutherland & Rudy,1989). Finally, Sakurai (1990) has suggested that the hippocampus acts as a temporary store that holds onto sensory information until it can be stored more permanently.

Thus, studies of hippocampal function continue to provide pieces of a puzzle, although the entirety of that puzzle continues to elude researchers. The fact that the hippocampus as an area of study continues to generate a great deal of interest suggests that scientists continue to see this as an interesting problem, which will hopefully soon be assembled to provide a full and accurate explanation of function.

Chapter 2: Hippocampal Structure

The hippocampus is a part of the limbic system. This important "border" between the cerebral hemispheres and underlying brainstem structures includes (at least) the cingulate and parahippocampal gyri, the septum, amygdala, hypothalamus and hippocampus. The limbic system is also known as the visceral or emotional brain, due to its involvement in behavioral and emotional expression. The limbic system receives inputs from sensory and associational sources, and additionally, has an impressive internal circulation of information. The hippocampus projects to the hypothalamus, the hypothalamus sends information on to the thalamus, which projects to limbic cortical areas that lead back to the hippocampus. This circular flow of information through the limbic system led Papez (1937) to suggest that this might be the neural mechanism of emotion.

The hippocampus is an integral part of the limbic system. It receives input from the parahippocampal gyrus, the septal nucleus, the hypothalamus, raphe nucleus, locus coeruleus, nucleus accumbens and the contralateral hippocampus. Outputs from the hippocampal formation project to the subiculum; from fimbria-fornix to septal nucleus, anterior hypothalamus and to the diagonal band of Broca (Bayer, 1985; Witter, 1986).

As noted in the connections described above, the hippocampus plays a role in linking the endocrine system and the brain. Recent discoveries have provided evidence that the effects of steroid hormones released by the adrenal cortex in response to stressful situations are mediated in the brain by at least two distinct receptor types.

The first type of receptor (Type I) is pharmacologically identical to the mineralocorticoid receptor found in the kidney. In the kidney, this receptor preferentially binds the mineralocorticoid aldosterone and is responsible for maintaining an appropriate balance of electrolytes (DeKloet, Ratka, Reul, Sutanto & VanEekelen, 1987). While it is also found in the brain, some of these Type I receptors function somewhat differently. In hippocampus, septal nucleus and dentate gyrus the Type I receptor preferentially binds corticosterone (CORT), also binds aldosterone with high affinity, and binds synthetic glucocorticoids with low affinity. This mineralocorticoid receptor is found distributed in low levels elsewhere in the brain, where it behaves similarly to the version found in the kidney: it regulates salt appetite. The mechanisms and purpose of the apparently unique function of the Type I receptor in the hippocampus remain a mystery.

The second type of receptor is the classical glucocorticoid (Type II) receptor (McEwen, 1987; Meaney, et.al. 1988). This receptor is widely distributed throughout the brain, and preferentially binds synthetic glucocorticoids such as dexamethasone (Reul & DeKloet, 1985). The Type II receptor appears to mediate negative feedback in the pituitary-adrenal system. In this closed-loop system circulating hormones feed back to affect further production and release of these substances (Keller-Wood & Dallman, 1984). It has been shown that glucocorticoid receptors have the capacity for auto-regulation. Chronic stress and exogenous application of CORT are both capable of reducing the number of CORT receptors in the hippocampus and amygdala, known as down-regulation (Sapolsky, Krey & McEwen, 1984). There is some evidence that the phenomenon of auto-regulation may result from the influence of hippocampal Type II receptors (Meaney et al., 1989). This evidence comes from studies in which rat pups are handled during the first 3 weeks of their lives. This manipulation produces an apparently permanent down-regulation of receptor concentrations in the hippocampus, accounted for by group differences in Type II, but not Type I receptors. Thus, the proportion of receptors in the hippocampus that are physiologically active at any

given time provides input to downstream systems about further release of glucocorticoids.

The hippocampus appears to provide influence at the hypothalamic and possibly at the pituitary level that lowers production of ACTH and its secretagogues; namely CRF, arginine vasopressin and oxytocin (Sapolsky, Armanini, Sutton & Plotsky, 1989). When the dorsal hippocampus is lesioned, exogenously administered glucocorticoids are no longer capable of reducing circulating CORT levels (Feldman & Conforti, 1980). Transecting the fornix results in hypersecretion of ACTH (Sapolsky et. al, 1989). These findings suggest that when input from the hippocampus to hypothalamus and pituitary is eliminated, CRF, ACTH and thus glucocorticoids continue to be pumped into the system at high levels during chronic stress and following termination of stressful events. Additionally, eliminating the production of glucocorticoids through adrenalectomy leads to increases in ACTH during stressful situations (Wilson, Greer, Greer & Roberts, 1980). Therefore, when there are no glucocorticoids present to bind to hippocampal receptors, the hippocampus provides no inhibitory signal to hypothalamus and pituitary, and high levels of CRF and ACTH result.

Thus, the hippocampus has structural features that suggest potential involvement in many functional capacities

- a fact that has generated a great deal of interest in this area, and simultaneously created confusion when a unitary theory is sought.

Chapter 3: Cognitive Functions of the Hippocampus

The fact that the hippocampus sits in a position to receive and transmit information to and from a variety of sources has led many scientists to question its functional significance. Lesions and alterations of the hippocampus suggest that this structure is indeed important in mediating many functions. A definitive understanding of the nature of hippocampal function continues to elude researchers, even though much is now understood about some of the functions known to depend on hippocampal influence.

It has long been known that the hippocampus is important for the acquisition and retention of some types of information. In humans, severe deficits in the ability to acquire new memories result from damage to the hippocampus. One now classic case, the bilateral surgical ablation of the hippocampus in the patient, H.M. produced a profound deficit in the ability to form new memories for any type of declarative information (Milner, 1966; Squire, 1987). H.M. can learn new skills, such as the solution to the Tower of Hanoi puzzle, although he does not remember learning the solution (Cohen, Eichenbaum, Deacedo, & Corkin, 1985).

Alzheimer's disease is a disorder in which deposits of plaque and tangled neural fibers impair increasingly large portions of the brain. The hippocampus, and entorhinal cortex are areas that are extensively damaged in Alzheimer's

disease (Van Hoesen, Human, & Damasio, 1991). Alzheimer's patients are unable to learn new information, such as word lists and new faces, although they are not impaired on learning tasks such as maintaining contact between a hand-held pointer and a moving object (Eslinger & Damasio, 1986).

Thus, the deficit seen in humans as a result of hippocampal damage can be characterized as either a problem in getting new information into memory, or an inability to retrieve information from storage. The ability to learn new procedural skills remains intact, suggesting a dissociation of these two forms of learning. Due to the difficulty of experimentally extending these findings in humans, animals are commonly used as subjects for studies of hippocampal function.

One obvious deficit produced by hippocampal lesions in rats is the impairment of spatial ability. This ability is often measured by means of the Morris water maze task, in which animals are required to learn about places based on cues distal to the goal. In order to escape from constant swimming in a tank fitted with a submerged platform, rats must learn the location of the platform from the cues provided by the room environment outside the tank. Because the water is made white, and the sides of the tank are white, the apparatus itself provides no cues as to the location of the escape platform. Thus, in order to learn

about the location of the platform, rats are forced to use the room itself, and the objects in the room to determine where they are within this environment, and how to navigate to the platform from their position. The water maze task essentially requires that rats form a cognitive map of the environment. The assumption is made that if a map has been established, an animal will swim directly to the platform after being released from any position along the wall of the tank. One reflection of map acquisition can be observed as a steady decrease in latency to climb onto the platform. Experiments that have used this task to measure the effects of hippocampal lesions reliably find that lesioned animals either fail to find the platform within the allotted trial time, or take longer than normal subjects to find the platform (Becker, Walker & Olton, 1980; Morris, Garrud, Rawlins & O'Keefe, 1982; O'Keefe, Nadel, Keightly & Kill, 1975; Rasmussen, Barnes & McNaughton, 1989).

Another popular means of measuring spatial behavior is the 8-arm radial maze, which takes advantage of rats' natural food foraging behaviors. This task requires that food deprived rats visit all 8 arms of the maze, without returning to any in order to obtain food. Efficient performance on the radial maze requires not only that the rat determine where in space a particular arm is located, but also that information be held about which arms have

already been chosen. So, the radial maze task contains a component that is similar to the water maze - recognizing places in space, but has an additional component - keeping in memory a running account of places already visited. Hippocampally lesioned rats fail to learn the radial maze task (Jarrard, Okaichi, Steward, & Goldschmidt, 1984; Olton & Papas, 1979). This fact has led some researchers to suggest that what the hippocampus contributes to cognitive function is not spatial per se, but rather, an ability to carry information across a time delay, or "working" memory. While this interpretation is more consistent with the findings of human studies, it does not rule out spatial learning as a function of the hippocampus; rather it extends potential functions.

Additional evidence for hippocampal involvement in spatial functions comes from studies where single cells are recorded as animals move through test environments. Cells, hypothesized by O'Keefe and Nadel (1978) as "place" cells respond to specific places in the environment. Electrophysiological studies have provided support for this hypothesis. In studies where firing of a single cell is recorded as a function of a particular location on a maze, it has become clear that there are cells that respond preferentially to one location (Speakman & O'Keefe, 1986). This relationship may depend on movement through the

environment (Foster, Castro & McNaughton, 1989; Muller & Kubie, 1989). Place cells are quiet while an animal is restrained, but fire in response to movement toward a certain location. These findings support the involvement of the hippocampus in responding to spatial components of the environment.

Thus, although specific demands of tasks used and the motivational conditions under which spatial memory is assessed varies between paradigms, the finding that the hippocampus is important for spatial learning remains constant.

The hippocampus is also involved in regulating exploratory behavior and general activity levels. When exposed to a novel environment, rats show little interest in most forms of behavior until they have explored and habituated to the new environment. New places may contain dangerous objects or events, or they may provide new opportunities to obtain needed resources. Rats have a typical pattern of behavior when placed into novel environments. They initially engage in what are interpreted as fearful forms of behavior - they freeze, defecate or attempt escape (Chozick, 1983; Takahashi, et al, 1989). Following this behavior, most rats engage in exploratory behavior: sniffing and traveling through the new environment; presumably to obtain information. Lesions to

portions of the hippocampus disrupt exploratory behavior. Woodmice failed to show normal goal alternation when lesions were made to entorhinal cortex, and they did not make prolonged visits to new objects placed into their environments. The decrease in the amount of exploratory behavior in this study was not related to a general increase in activity. Lesioned animals were observed to engage in MORE locomotion and wheel-running than control animals (Schenk, Inglin & Gyger, 1983). Gerbils have also been observed to display decreased amounts of object contact following hippocampal lesions (Glickman, Higgins & Isaacson, 1970). These studies suggest that the deficits observed in hippocampal animals in novel environments are specific to the information-gathering, or exploratory portion of behavior in novel situations.

While these studies provide evidence that lesioned animals are engaging in less exploratory behavior, care has not always been taken to separate exploratory behavior from general increases in activity levels. This is due in part to the finding that damage to the hippocampus leads to hyperactive behavior (Fass, 1983, Schenk, et al., 1983). Thus, lesioned animals may be engaging in more movement, but actually exploring less. Recent attempts have been made to separate the two forms of behavior. Renner (1987) measured locomotor movements in rats exposed to different

environments, and additionally collected detailed information about the number and type of interactions subjects made with objects in a test arena. These behaviors were differentially affected by the manipulation used - a finding that suggests that activity and exploration are different forms of behavior, and that they may be mediated by different mechanisms. Additional support for the separability of activity and exploration is provided by a principal-components analysis conducted by Maier, Vanderhoff & Crowne (1988). These authors collected data from an emotionality rating scale, running-wheel activity, an open-field measure, novel object detection, and water maze performance. Sixty-five normal rats provided the data and the analysis showed that activity and exploration loaded as separate factors (exploration and emotionality however, were not easily separated).

Thus activity and exploration are separate forms of behavior, both of which are related to the hippocampus, and interestingly, both of which are influenced by adrenocortical activity.

Rats have an adrenocortical response - i.e. an increase in CRF, ACTH, and corticosterone, when placed into a novel environment. This response habituates after about 4 hours in a single exposure, and after five days of repeated exposures (Pfister, 1979). Direct manipulations of the

adrenocortical system affect activity and exploration. In one study, a CRF antagonist resulted in an increased willingness of rats to quickly enter an open-field, whereas CRF administered into the brain, made rats less likely to enter the open field (Takahashi, Kalin, Burgt & Sherman, 1989).

A possible explanation for the relationship between activity, exploration and adrenocortical activity is that the hippocampus receives multimodal sensory inputs, which either form a match with some stored representation, or fail to form a match. The failure to match an existing spatial map produces the experience of novelty - thus an adrenocortical response is initiated. It may be that the detection of a novel situation, with its elicited adrenocortical activity regulates the typical pattern of behavior observed in rats responding to new places. Lesioning the hippocampus would interrupt typical behavior in one or all of the following ways: the animal may fail to recognize familiar places, may not attempt to gain information (explore) if that information cannot be integrated with past knowledge, may fail to produce, or alternatively to terminate an adrenocortical response.

Chapter 4: Emotional Functions of the Hippocampus

Emotion is a form of motivation. Animals seek and engage in behaviors that produce pleasurable results. They avoid behaviors that produce pain, run away from fearful situations, and act in ways that apparently reduce anxiety. Darwin (1872) suggested that emotions are biologically wired, are behaviorally exhibited, and are adaptive. Thus, anger, fear and even joy are responses to events in the world that contribute to an animal's fitness and survival.

Most theories of emotion are based on the premise that three components combine to produce emotion: physiological arousal, a cognitive factor and some form of behavioral expression (Buck, 1984; Izard, 1989; Plutchik, 1985). Although there is disagreement as to the exact order in which these components occur, and the influence each has on the others, that all are necessary to the experience of emotion is not contested.

The physiological arousal associated with emotion involves activation of the sympathetic nervous system. Increases in heart rate, respiration and glucose availability, dilation of the pupils, and decreases in nonessential functions (such as digestion and reproduction) all serve to prepare an animal to respond appropriately to challenges from the environment. The activation of the sympathetic system comes from the hypothalamus which

is the link in the adrenocortical system that responds to descending inputs by releasing corticotropin releasing factor (CRF) to the pituitary. In 1937, James Papez proposed a neural mechanism for emotion: essentially a loop through the limbic structures. The hippocampus sends inputs through the fornix to the hypothalamus. Hypothalamic projections to the thalamus are directed to limbic cortex, and finally back to the hippocampus through the fornix (Angevine and Cotman, 1981).

Thus, one effect of challenging events is that they produce physiological arousal in the peripheral nervous system. A second component to emotion is the cognitive interpretation of events that produce arousal. Clearly not all stimuli activate the autonomic nervous system, and a single stimulus sometimes produces arousal, and sometimes fails to arouse. While an encounter with a bear in the woods is likely to activate physiological arousal, a bear in the zoo usually does not. Thus, an organism must recognize sensory information as threatening, novel or associated with pleasurable consequences in order to elicit a physiological response. As discussed in the previous chapter, the hippocampus is involved in learning about spatial aspects of the environment, and about the context in which events occur - exactly the sort of cognitive interpretation necessary for an emotional response to occur.

A third component of emotion involves a behavioral response to challenging events. An animal that is afraid may run, fight or freeze. Frustration may produce a species-typical aggressive response (Dollard, Doob, Miller, Mower & Sears, 1939). Animals repeatedly engage in behaviors that produce pleasurable results. Although as mentioned earlier there is a great deal of disagreement as to which component of emotion causes the others to happen, behavior is the most easily observed, and particularly for the rat, the most commonly studied.

It can be argued that the hippocampus plays some role in all aspects of an integrated emotional response. First, a cognitive component, or initial detection of a situation that requires a response must occur. The hippocampus, due to its involvement in novelty detection, determining contextual relations and mediating spatial knowledge seems to provide the impetus for an initial state of physiological arousal that accompanies emotional states. The glucocorticoid receptors localized in the hippocampus are also responsible for terminating the physiological response. It could also be argued that the hippocampus contributes a component to the behavioral expression of emotion. It is known that novel places elicit an increase in activity levels and exploratory behavior in rats (Denenberg, 1967). Under certain circumstances, rats are also likely to freeze

when placed into new environments; a response that is of considerable adaptive value in the real world (Chozick, 1983). Not only are these behaviors altered when the hippocampus is lesioned, but they are also affected by interference with normal levels of stress hormones (Pfister, 1979). Inasmuch as physiological arousal occurs as a response to novel situations, and exploration and freezing are behaviors observed in novel situations, and all of these components of emotion are known to be influenced by the hippocampus, it seems reasonable to suppose that this structure is serving some sort of a linking function between the separate features.

Chapter 5: Neuroendocrine Functions of the Hippocampus

The body's slow-acting, long range messenger system provides much of the modulatory influence responsible for keeping physiological systems within some genetically determined range. Body temperature, blood sugar levels, salt balance and circadian fluctuations of sleep/wake cycles are examples. The neuroendocrine system is also responsible for altering physiological functions as dictated by current needs of the organism, such as would be the case when changing environmental conditions necessitate a reaction in order to insure safety or provide for the animal's current needs. More specifically, the hippocampus provides the negative feedback that terminates the hormonal response to stressful situations. A second critical endocrine aspect of hippocampal involvement in stress is less well understood. This aspect relates emotional state and learning: organisms exposed to novel or potentially threatening situations may well learn different things than when they are in more familiar surroundings. Two aspects of the neuroendocrine system rely heavily on hippocampal input. First, initial activation of autonomic activity may be directed by hippocampal inputs to the hypothalamus (Nauta & Feirtag, 1991). Second, the hippocampus provides the negative feedback that terminates the hormonal response to stressful situations (Meaney, 1988). Thus the hippocampus appears to

have some control over both activation and maintenance of the neuroendocrine response to environmental conditions.

Chapter 6: Effects of stress on learning and memory

What an organism learns during a stressful experience may differ quite a bit from that which is learned during non-stressful situations. As suggested above, there are many concomitant alterations in the hormonal and neurochemical environment during activation of the stress response system. There have also been detailed studies of the effects of hormonal alterations on learning and memory functions, some of which will be discussed here.

A. Catecholamines

Adrenaline and noradrenaline are produced peripherally in the adrenal medulla, and released from that site in response to arousing or stressful situations (Dunn, 1980). In humans, adrenaline levels have been shown to be elevated in urine samples during times of anxiety and fear, while noradrenaline is more related to events in which anger and action responses dominate (Elmadjian, 1959; Goodall, 1962). Interestingly, high levels of peripheral adrenaline depend on the action of glucocorticoids. When these steroid hormones are released from the adrenal cortex, they travel through the adrenal portal system and induce synthesis of an enzyme (PNMT) that converts noradrenaline to adrenaline (Wurtman, Pohorecky & Baliga, 1972). Thus, peripheral levels of catecholamines are related at least to some extent

on centrally generated messages concerning interpretations of the environment.

Centrally, adrenaline is produced in the medulla oblongata and the reticular formation. From these sources, adrenaline is sent to thalamic and hypothalamic sites, and also descends to spinal cord destinations. Noradrenaline is produced primarily in the locus coerulus and travels to the medulla and pons areas. The fibers of this system proceed ventrally to form connections with the medial forebrain bundle. From this area, fibers fan out to innervate limbic and cortical structures (McGeer and McGeer, 1980).

While catecholamines have an important role in the immediate response of the body for the mobilization of the system for flight or action, they also influence learning and memory. According to a review by Adrian Dunn (1980): "In general, antagonists of catecholamine metabolism interfere with learning and memory, whereas agonists can improve it" (p. 376). An important point to be considered here is evidence which suggests that the effects of catecholamines on learning and memory are related to peripheral rather than central release of these substances (McGaugh, 1980). Although little is understood about the relationship of catecholamines to learning and memory, there is some evidence to suggest that the peripheral arousal that

accompanies stress and emotion (and is related to learning) is mediated by peripheral catecholamines.

B. ACTH

The administration of ACTH had been shown to alter the acquisition and retention of passive avoidance tasks, and to inhibit extinction of aversive and appetitive tasks. In the classic paradigm first used by De Wied (1964), rats are placed into a 2-sided box. After being allowed to cross into the preferred dark side several times, the animals are given a shock upon entry, removed from the apparatus, and later tested on their latency to reenter the dark side of the box. Hypophysectomy (removal of the anterior pituitary) impairs retention in this task, but performance can be restored by a posttraining peripheral injection of ACTH. Although in this case ACTH serves as a replacement for missing endogenous levels, the enhancement of learning is also observed in intact animals (Beatty, Beatty, Bowman & Gilchrist, 1970; Gold & Delanoy, 1981). Adrenalectomy, which results in increased levels of ACTH, facilitates active avoidance at high, but not low shock levels (Beatty et al, 1970). Reducing ACTH levels results in an opposite effect. Following training on a passive avoidance task, retrograde amnesia was induced by immersion in cold water. Rats given a "reminder" treatment of recooling or shock showed retention of the passive response, except when

dexamethasone treatments were given (dex reduces circulating ACTH levels) (Santucci, Riccio & Treichler, 1989).

An additional effect of ACTH is an increase in conditioned responding during extinction trials. Compared with control animals, those treated with ACTH persist in responding for longer periods of time when responses are no longer reinforced (Guth, Levine & Seward, 1971). This effect is opposite to that seen with epinephrine or corticosterone, where extinction of passive and active avoidance are facilitated (McGaugh, 1983).

The behavioral effects of ACTH are thought to be mediated through extra-adrenal effects, that is they are due to direct effects of ACTH on the brain rather than through ACTH influence on the production or release of adrenal hormones. There are ACTH binding sites at several locations in the brain (Krieger & Liotta, 1979), and ACTH injected directly into cerebral ventricles affects learning at much lower doses than those needed when injected peripherally (de Weid et al., 1978). Adrenalectomy, which results in increased ACTH levels produces results similar to exogenous administration of the hormone, and analogs of ACTH that have no steroidogenic properties also produce the effect (McGaugh, 1983).

There are several possible interpretations for these findings. First, it may be that ACTH acts to increase

motivational and attentional properties of environmental events (Bohus, 1973). It has also been suggested that ACTH levels at the time of training provide a state-dependent retrieval system for potentiating responses when retested later. That is high levels at both times produce a congruent state that facilitates retrieval (Santucci et al., 1989). Finally, it has been suggested that ACTH exerts its effects through interactions with other hormonal systems, notably norepinephrine (Gold & Delanoy, 1981) or through CORT interactions (DeKloet, Veldhuis & Bohus, 1980).

C. CORT

The literature concerning effects of steroid hormones on learning and memory must be interpreted carefully for two reasons. First, much of the experimental evidence for involvement of these hormones in learning predates knowledge of the dual receptor system. Thus, effects of glucocorticoids such as cortisol and corticosterone, and dexamethasone are not distinguished from those produced by mineralocorticoids such as deoxycorticosterone and aldosterone. Although many studies used substances from both groups and found opposing effects, it is difficult to interpret these findings. This is due to the fact that dexamethasone nearly always binds to Type II receptors, and mineralocorticoids can be assumed to bind exclusively to Type I receptors, but corticosterone binds to both in

amounts related to circadian variation. A second problem concerns differential occupation of the two receptor types, and the confound produced by circadian fluctuations and endogenous alterations of occupation, such as occurs with food deprivation. In rats (and humans), a circadian surge of glucocorticoids occurs just prior to waking, then drops to lowest levels at normal sleep times (McEwen & Brinton, 1987). This hormonal surge is accompanied by increased exploration and food-seeking behavior. It would seem appropriate to test laboratory animals during the time when they are hormonally most active. The problem is that rats are nocturnal, and their days and nights are regulated by laboratory light/dark cycles, thus the time when lights go off in the lab (usually at night) is the time when rats are at high hormone levels. Unfortunately, behavioral testing is usually done in the morning or afternoon, during the animals' normal sleep time. Even more of a problem is that prior to the mid-80's few papers reported the light/dark arrangements of the animal room where experimental subjects were housed.

An early study examined the effects of prestressing an animal prior to testing for level of avoidance responding. This pretraining shock would presumably activate the endogenous hormone levels of an animal and produce a somewhat natural activation level of the stress response

system (Weiss & Gray, 1973). The experiment provided evidence that prestressed animals responded at higher rates than unstressed controls. Adrenalectomy abolished the prestress effect, while hypophysectomy and adrenal demedullation had no effect. The effects of adrenalectomy could be abolished by the administration of deoxycorticosterone or aldosterone, but not by dexamethasone. Additionally, intact rats maintained on a 1.5% saline drinking solution, which greatly reduces endogenous aldosterone levels showed no effect of the prestress manipulation. These findings provide evidence for involvement of the Type I (mineralocorticoid) receptors in mediating behavioral responses to stressful stimuli.

Other evidence implicates Type II receptors in the mediation of behavioral phenomena. Normal rats placed into a water-filled cylinder will gradually decrease activity over a learning trial, and upon return to the tank will maintain immobility at about 70% (Jeffereys et al., 1983). Adrenalectomized rats fail to acquire immobility in this task (CORT effects), hypophysectomized animals behave like control rats (not ACTH), and animals that are both adrenalectomized and hypophysectomized behave like adrenalectomized only animals (ADX not attenuated by HYPO). The ADX deficit can be remediated by administration of corticosterone, dexamethasone or met-enkephalin, but not by

deoxycorticosterone. These findings suggest that elevated steroid hormone levels are responsible for the retention of some learned responses, and that the effects of acquired immobility are probably due to activation of Type II receptors.

Acquisition and extinction of avoidance responses have been used to study effects of various hormones on learning and memory. In one study adrenalectomized animals showed an impaired passive avoidance response, indicated by decreased avoidance latencies to reenter a dark compartment in which they had been shocked earlier (Borrell, DeKloet & Bohus, 1984). Retention could be facilitated by administration of adrenaline at moderate, but not high doses, probably reflecting the inverted U-shaped curve of dose response generally seen in drug effects. Corticosterone had no effect when administered alone (it didn't restore passive avoidance to normal levels), but CORT did abolish the effects of moderate doses of adrenaline. At high adrenaline doses, which had previously proved ineffective in restoring avoidance responses, the addition of CORT rendered the dose effective. Dexamethasone, like CORT had no effect when administered by itself to the ADX animals, and when given in conjunction with adrenaline produced results similar to those observed with adrenaline alone. The authors interpret these findings to mean that post-learning events are

modulated by adrenaline, and that corticosteroids act to eliminate responses that are no longer relevant. They also suggest that CORT is a mechanism designed to protect an organism from stress responses, including high levels of adrenaline that would damage neural processes. CORT may exert its influence by interacting with adrenaline to reduce its efficacy.

Whereas the previously described study showed an impairment in the acquisition of passive avoidance, another study found that adrenalectomized animals failed to extinguish a passive avoidance response (DeKloet, Velduis & Bohus, 1980). The important part of this study was the finding that extinction of the response could be normalized by the administration of CORT, but not progesterone or dexamethasone. This points to involvement of the Type I receptors in mediating extinction of passive avoidance responses. The contradictions contained in these studies suggest that acquisition, retention and extinction may be phenomena mediated by different biochemical elements.

Corticosterone has also been implicated in an appetitive extinction task, where ADX rats demonstrated a more rapid and prolonged extinction of runway running for a food reward (Micco, McEwen & Shein, 1979). This result is reminiscent of retardation of extinction seen in hippocampally lesioned rats (Jarrard, Isaacson & Wickelgren,

1964), and suggested to the authors that the hippocampus may be involved through the adrenal steroid system in hippocampally mediated tasks. Thus, these studies assessed the contribution of hormones to behaviors known to be mediated by the hippocampus (Micco & McEwen, 1980). Neither spontaneous alternation nor habituation of exploration was affected by adrenalectomy or administration of CORT. Hippocampally lesioned animals alternated less, and had higher levels of activity and exploration, neither effect was remediated by the administration of CORT. However in an appetitive extinction task, ADX animals had faster extinction, and this result could be normalized by CORT, but not by DEX. The authors suggest that the hippocampus is involved in the inhibition of response, so when its influences are removed (or it is hyperactive) extinction slows because the animal cannot inhibit a learned response. Another possibility for these findings, the authors suggest is that the degree to which learning is involved is important, and that the need for behavioral change may be a factor necessary to invoke pituitary-adrenal activation.

Another study which attempted to relate hippocampal behavior to adrenal hormones similarly failed to find direct effects. Adrenalectomized rats trained to find food on each of 8 arms of a radial maze showed no deficits in their ability to solve the task at retention intervals up to 24

hours (Yongue & Roy, 1985). This suggests that the steroid hormones produced in the adrenals are not critical modulators of this task. The interpretation of the authors is that win-shift strategies used by rats to solve this task are well-known, unlearned responses, and again, that glucocorticoids may affect behavior only when qualitative changes in behavior are demanded.

Thus it seems that many behaviors are affected by hormones produced in the adrenal cortex, that the relationship is complex, and that the manner in which hippocampally dependent behaviors are influenced by this system is not easily characterized.

D. Conclusions

When stress effects are studied, what becomes immediately obvious is that all roads lead to the hippocampus. As noted above the endocrine and biochemical aspects of stress all affect and are affected by the hippocampus. The circadian variation of adrenal steroids is mediated by the hippocampus, through Type II receptors. A second, less well-understood system for binding steroids operates via the type I receptors, also found in the hippocampus. ACTH, adrenaline and noradrenaline all bind in the hippocampus.

Evidence implicates the hippocampus in emotional function. Although it probably isn't responsible for the

expression of fear per se, the hippocampus seems involved in responses where conflict arises from unknown situations, or those in which there is disparity between what is known or expected, and reality. This definition refers to what we know as anxiety, which is a component of stressful situations.

Finally, studies of the effects of hormonal manipulations on many forms of learning suggest that the endocrine involvement of the hippocampus must play a role in the mediation of many forms of learning. Avoidance responding, extinction and acquired immobility are all forms of learning that are affected by increased or decreased levels of ACTH and corticosterone. The final piece of evidence required to nail down a theory of hippocampal mediation of stress response is support for steroid hormone effects on hippocampally dependent behavior. However, research to date has shown these behaviors are not affected by steroids in any immediately obvious manner. Exploration, spontaneous alternation and spatial learning are not affected by adrenalectomy. These findings require explanation. Micco and McEwen (1980) suspected that exploration and alternation were forms of behavior that did not produce high levels of "task activation", or stress, and were thus not affected by the loss of adrenal steroids. They also hypothesized that some form of behavioral change

from an animal's normal, innate response might be required to elicit pituitary-adrenal effects. Yongue and Roy (1985) interpreted their lack of adrenalectomy effects on spatial learning as a result of the fact that win-shift strategies are the rat's preferred method for solving appetitively motivated tasks. They go on to suggest that well learned or innate responses are not affected by adrenal hormones, and like Micco and McEwen (1980), thought circumstances that required an animal to deviate from its accustomed response pattern might be likely to require hormonal mediation.

Continuing along these same lines, it might be perfectly reasonable that the hippocampus serves as a stress response system, AND that the behaviors that are mediated by this structure are not immediately influenced by fluctuations of stress-related hormones. Hippocampal behaviors, exploration, alternation and spatial learning are all important responses of an animal to novel, possibly threatening environments. The time required for activation of the adrenal secretion of catecholamines, glucocorticoids and mineralocorticoids is not sufficient for the immediately adaptive responses that promote survival. Thus if these behaviors were mediated by a system that had early access to information about the environment, like the hippocampus, these behaviors would be activated anytime an animal perceived an unknown or threatening situation. This

that they had been using a turning response strategy, most of these ignored the food which was in the wrong place and quickly ran down the opposite arm and ate food found in the accustomed place (Wilson, unpublished observations). This suggests that the animals had learned something about the place where food was found, but that once having learned this, another (less costly?) strategy replaced the place response. In a similar study where the rats were "probed" at an earlier stage of training, normal young adults almost exclusively displayed a place strategy for solving the task (Barnes, Nadel & Honig, 1980).

Exploration of novel environments is also a hippocampally mediated behavior that changes over time. Normal rats engage in high levels of exploratory behavior when first introduced to novel environments, followed by habituation of exploration. Hippocampally lesioned rats engage in much activity in novel environments, but there is reason to suspect that this activity is not exploration, but merely increased activity, and lesioned rats fail to habituate activity levels. In the Micco and McEwen (1980) study reported above, hippocampectomized rats displayed increased activity levels across the 10 minute test period, an effect no observed in adrenalectomized animals, with or without CORT replacement. This finding suggests that the hippocampus, but not adrenal steroids are involved in

mediating activity/exploration levels of rats placed in novel situations.

Thus, there is evidence to support the idea that the hippocampus mediates the response of an animal to stressful situations in two ways. First through species-specific adaptive behaviors that are mediated by this structure, and second by orchestrating the endocrine responses that are processed through the hippocampus.

Chapter 7: Integration of function

Taken together, the involvement of the hippocampus in cognitive, emotional and endocrine activity suggests that this structure provides a means by which external events influence the internal mobilization of the body to respond appropriately to these events. If this is the case, then it would be expected that disruptions of the hippocampal system would result in severing the connections between external stimuli and the organism's response to these stimuli. How then, does this fit with the suggestion that the hippocampus mediates an animal's adaptive response to the changing environment in which it must survive? Adaptive responses necessarily require the collection and integration of information from a wide variety of sources. Clearly learning, emotion and endocrine activity all exist in some form in the absence of the hippocampus. What is not as clear is how the removal of hippocampal influence may affects the ways in which an animal takes in and uses all dimensions of environmental information. Surviving in the real world involves more than piecemeal responses to discrete stimuli - many bits of information must be pulled together to provide solutions to complex problems. The hippocampus may be doing exactly this as it combines cognitive bits into maps, emotional bits into appropriate

responses, and directs mobilization of the entire system through its neuroendocrine connections.

Chapter 8: Development

Although a great deal of development takes place prenatally, the young of many species are born before reaching complete maturity. Not only does this imply that the immature organism will most likely require some degree of parental care and nurturing, it also means that the infant will be exposed to more fluctuations of the environment than it experienced while it developed within the relatively protected uterine environment. Temperature, food supply, exposure to predators, and quality of parental care are some of the things that may differ among young animals. Thus, young organisms are exposed to variations in experience, but for most, there is protection from many events that mature animals must confront. There is also opportunity for many behaviors to develop in a parentally protected environment. Young animals can and do play at behaviors that will be essential to survival later: chasing, pouncing, sexual behavior and exploration are examples. Experience that deviates too much from what is expected sometimes results in abnormal development. This is particularly obvious in systems that undergo extensive postnatal development. One such structure is the hippocampal formation, which completes much of its development postnatally. This postnatal development can be influenced by varying environmental conditions during the

critical early period of life. As will be discussed below, handling and isolation are forms of experience that can alter hippocampal development.

A. Structure

The hippocampal glucocorticoid receptor system undergoes significant postnatal maturation, and various forms of early experience may exert some of their effects by altering the final number or concentration of these receptors. This appears to be one of the effects of early handling in rat pups. It is unclear whether isolation of rat pups from their mother interferes with development of glucocorticoid receptors, but there is some reason to suspect that this may be the case. There are also ontogenetic changes in receptor binding for various neurotransmitters (Baudry, Arst, Oliver & Lynch, 1981). Finally, the glucocorticoid receptor system known to be highly concentrated in the adult hippocampus is engaged in a phase of rapid development during the early postnatal period. Recent work indicates the presence of two distinct receptor types (Rosenfeld, Sutanto, Levine and DeKloet, 1988; Sarrieau, Sharma & Meaney, 1988), each with a unique developmental pattern (Rosenfeld et al., 1988). The mineralocorticoid receptors, which in the central nervous system are confined primarily to the septohippocampal system and pituitary are undetectable during the first few days

following birth. These receptors can be measured at adult levels by 8 days of age in the rat, and undergo very little change after that time. The classical glucocorticoid receptors, which are widely distributed throughout the brain, are present at birth. The developmental changes of these receptors are primarily related to affinity and concentration. As noted earlier, in adult rats, mineralocorticoid receptors bind corticosterone (CORT) with high affinity, while classical glucocorticoid receptors bind CORT with low to moderate affinity. During the ontogenetic absence of mineralocorticoid receptors, classical glucocorticoid receptors bind CORT with high affinity. This high classical glucocorticoid affinity begins to decrease when mineralocorticoid receptors "turn on" somewhere between 4 and 8 days and reaches adult levels by day 8 (Rosenfeld, et al, 1988). Concentration changes are also evident in the classical glucocorticoid receptors. At 2-4 days immunoreactive staining shows the receptors distributed in the pyramidal cells of all hippocampal regions, CA1-CA4, and in the granule cells of the dentate gyrus. Immunoreactive staining decreases in all areas, and completely disappears from CA3 and CA4 by day 12. Evidence of increased staining is seen in CA1 and CA2 and the dentate gyrus at day 16, and reaches adult levels by day 20 (Rosenfeld, Van Eekelen, Levine & DeKloet, 1988). The results of this recent work

have led both Rosenfeld and her colleagues, and Sarrieau's group to suggest that developmental plasticity of the classical glucocorticoid receptors may be responsible for the effects obtained through manipulations of the early environment.

B. The Development of Behavioral Functions

Large domains of behavioral function emerge after birth in altricial species such as the rat, monkey and human. It is becoming clear that at least part of the reason for these delays lies in the postnatal maturation of various brain regions. The hippocampal formation includes the hippocampus proper and the dentate gyrus, the latter having been the focus of considerable attention in the developmental neurosciences. It contains about 600,000 cells in the mature rat, over 80% of which are generated postnatally, during the first two weeks of life (Schlessinger, Cowan & Gottleib, 1973). Most synapses in the rat dentate gyrus are formed postnatally, essentially adult levels being attained by postnatal day 25 (Crain, Cotman, Taylor & Lynch, 1973). Data from physiological studies converge with these anatomical findings: "theta" activity in the hippocampal EEG, mature synaptic function, and long-term potentiation (LTP) are only seen after 2-3 weeks in rats (LeBlanc & Bland, 1979; Creery & Bland, 1980; Wilson, 1984).

There is some evidence from behavioral studies that

suggests a linkage between this structural maturation and the emergence of forms of behavior thought to be dependent on the hippocampus (Somerville, 1979). Thus, studies of exploration, spontaneous alternation, and spatial learning demonstrate an absence of these functions until the third or fourth week of life (Feigley, Parsons, Hamilton & Spear, 1972; File, 1978; Kurz, Harkins & Nadel, 1984; Rudy, Stadler-Morris & Albert, 1987).

Chapter 9: Early Experience

A variety of early experiences has been shown to alter behavior, and to produce morphological changes in animals. Two manipulations pertinent to this proposal are handling and isolation. Each will be further discussed here.

A. Handling

Young rat and mice pups that have been handled prior to weaning have generally been shown to be less emotional as adults, to demonstrate increased learning capacities, and to exhibit neural differences throughout life (see Daly, 1973 for a review).

Emotionality has usually been measured by activity in an open field and by defecation, with the interpretation that less emotional animals defecate less and move around more (Daly, 1973). Generally, handled animals defecate less (Levine, 1959; Denenberg, Rosenberg, Haltmeyer & Whimbey, 1969; Denenberg & Morton, 1962) and are more active than nonhandled controls (DuPreez, 1964; Denenberg, et al., 1969). In addition to this behavioral evidence of reduced emotionality, it has been found that handled rats display reduced pituitary-adrenal response when subjected to cold stress (5 degrees C.) for 90 minutes (Levine & Lewis, 1959; Levine, 1962), and that handled rats have more glucocorticoid receptors in the hippocampus (Meaney, Aitken, Bodnoff, Iny, Tatarewicz & Sapolsky, 1985).

Although handling in infancy appears to alter emotional responsivity, the results of studies to determine differential learning abilities are equivocal. Avoidance learning and tasks involving noxious stimuli show handled animals to be superior to nonhandled animals (Chevalier & Levine, 1955; Du Preez, 1964). A simple maze running task found no difference in running speed between handled and nonhandled animals (Spence & Maher, 1962). Wong and Jamieson (1968) found facilitatory effects of handling on discrimination and reversal learning, while Wong (1966) found no effects of handling on acquisition or extinction in a T-maze. In a study using mice, Smith (1967) found handling to improve performance on brightness and pattern discrimination tasks, but to impair performance on a spatial task.

These findings suggest that some, but not all, forms of learning are affected by handling. As many authors have noted the performance of animals on learning tasks is affected by their emotional reaction to the test situation (Daly, 1973; Smith, 1967; Chevalier & Levine, 1955; Spence & Maher, 1962). As handled animals react to novel situations with more willingness to explore, it might be expected that tasks related to movement through the environment would be most affected by handling. Surprisingly this is not the case. Tasks enhanced by handling include avoidance

learning, those involving noxious stimuli and several varieties of discrimination. Running speed on a simple runway and acquisition and reversal of a T-maze showed no effect of handling, and spatial learning was impaired by handling. Thus these results suggest an effect of handling on learning, but the nature of the effect has not been clearly defined.

The histological assessment of the effects of handling on neural development presents a rather more reliable picture than that of learning. There appears to be an increase in glial cell numbers in the anterior commissure and indusium griseum, as well as an increased number of myelinated axons in the anterior commissure of handled animals at 6 months of age (Sturrock, Smart & Tricklebank, 1983). Brain weights of handled animals are lower than nonhandled controls, as are areal measurements of the cerebellum, neocortex and hippocampus. Cell proliferation in these areas is at first slower in handled animals, but then increases to exceed that found in nonhandled animals (Altman, Das & Anderson, 1968). Both of these studies suggest that there is an initial retardation of brain growth, followed by a prolongation of neural development. The result is differences in adult neural structure which may account for functional differences observed in adult animals.

Another proposed mechanism for the effects of handling in infancy is differential development of the glucocorticoid receptor system. Dexamethasone (a synthetic glucocorticoid) binding has been found to be higher in the hippocampus and frontal cortex of handled animals than nonhandled controls (Meaney, Aitken, Bodnoff, Iny, Tatarewicz & Sapolsky, 1985). The classical glucocorticoid receptor binds with high affinity to dexamethasone, while the mineralocorticoid receptors bind with only moderate affinity, leading to the conclusion by the authors of this study that these (classical glucocorticoid) receptors are the ones affected by handling. Interestingly, while this receptor is found throughout the brain, the increase in receptor numbers due to handling is limited to the hippocampus and frontal cortex.

Thus, although the experience of handling during infancy may produce lasting changes in the structure and function of the brain, it is still unclear exactly what these changes may be and which feature of the environment may be responsible for their mediation.

B. Isolation

Although the effects of handling on neural development have frequently been attributed to stress of the infant (cf. Altman et al, 1968; Meaney et al, 1985), studies that have sought to provide evidence of increased levels of

glucocorticoids in stressed infants have found little evidence for this. Events that normally produce elevations of corticosterone in adult rats, such as surgery, ether, handling and thermal disruption, often fail to have the same effect on infants (Sapolsky & Meaney, 1986). Thus it would appear difficult to elicit a pituitary-adrenal response in rats during the first two weeks of life. This is important because when an increase in circulating levels of glucocorticoids is induced by the administration of cortisol, corticosterone or dexamethasone, cells that are undergoing mitosis slow or halt division (Bohn, 1985). One manipulation that has been shown capable of increasing the normally low levels of corticosterone in rat pups is isolation from the dam (Stanton, Wallstrom and Levine, 1987). Following a period of at least 8 hours of separation, rat pups show a significantly elevated level of corticosterone when exposed to novelty stress (Stanton and Levine, 1989; Stanton, Wallstrom and Levine, 1987). The long range effects of this manipulation on neural organization are not clear, but at least two possibilities exist. First, there may be a reduced number of late developing cells. Second, there may be an alteration in the number or concentration of glucocorticoid receptors.

Studies to date which have explored the behavioral effects of isolation in infancy have concentrated on social

and emotional changes. Harlow's classic studies of monkeys reared in isolation indicate that these animals have severe social and emotional deficits in adulthood (Harlow & Zimmerman, 1959; Hinde & Spencer-Booth, 1971). Dogs have also been observed to display aberrant emotional behavior following severe sensory deprivation during development (Melzack & Thompson, 1956). Assessment of learning abilities in socially isolated monkeys demonstrated no deficits in general learning ability (Harlow, Harlow, Schlitz & Mohr, 1971). However, it should be noted that the tasks used by Harlow et al; discrimination, delayed response and oddity learning set, are not known to be hippocampally mediated and therefore would not be expected to be deficient if interference with development of the hippocampus is the major effect of isolation in animals. Far less is known of cognitive, specifically hippocampal, function in rats that have been isolated during development.

Handling and isolation appear to be two forms of experience that alter neural development when administered to neonates. The commonalities between these two events is not immediately obvious. However, both apparently exert at least some of their effects by way of the pituitary-adrenal response system, of which the hippocampus is an important link. Handling results in an increase in Type II glucocorticoid receptors in the hippocampus with a

concomitant enhancement of negative feedback in the stress response system. Although handling appears to have a facilitatory effect on general learning abilities, there is a suggestion that hippocampal tasks may be impaired in handled animals (see Smith, 1967). Isolation, on the other hand produces an elevation of normally low levels of glucocorticoids in infant rats. Although it is unclear what the long-term anatomical results of isolation may be, synthetic glucocorticoids administered perinatally result in decreased size of the hippocampus and cerebellum, and in deficits in hippocampally mediated tasks (Vicedomini et al., 1986). Finally, the combination of isolation and handling has been shown to impair performance on hippocampal tasks (Nadel & Willner, 1990).

Thus handling and isolation are both forms of early experience that may alter behavioral functions of the hippocampal formation, probably through alterations of the glucocorticoid response system. It has long been known that arousal levels affect performance (Hebb, 1955). What has been lacking until now is some understanding of the neural mechanisms responsible for mediating this relationship. As discussed above, the dual role of the hippocampus in emotional and cognitive functions suggests that it may be a plausible candidate for the neural link between arousal and performance.

Chapter 10: Experimental hypotheses

The present study explored the relationship between emotional and cognitive components of hippocampally mediated behavior. More specifically, this research tested the hypothesis that both handling and isolation are forms of experience that affect the developing hippocampus. Learning was assessed with several tasks, some which are known to depend on the hippocampus, some not. If hippocampal learning is affected by early experience, it was predicted that the handled, isolated, and handled/isolated animals would perform poorly on a hippocampally task, but not the control tasks. If emotional differences are the main effect of the manipulations, these emotional factors may interfere with learning. If this is the case, then experimental animals were expected to perform differently from control animals on all learning tasks. In order to correlate learning indices with emotional factors, activity in an open field was used as an indicator of each animal's response to a novel situation. It was expected that increased activity would not be positively correlated with acquisition of spatial learning, in fact it was suspected that the opposite would prevail, i.e. there would be a negative correlation between activity and spatial learning. When the task was nonspatial, however, it was expected that there might be a positive correlation between activity and learning.

The measurement of corticosterone (CORT) levels was somewhat exploratory in nature. Based on the studies of Meaney et al. (1985; 1988a; 1988b; 1989), it was expected that handled animals would have decreased CORT levels. There are no predictions from the literature concerning adult levels of CORT in isolated animals, but based on behavioral measures, it was suspected that CORT levels would be altered. This prediction arises from the finding that maternal separation of rat pups elevates CORT at a time when it is normally at very low levels (Stanton, Wallstrom & Levine, 1987; Stanton & Levine, 1989). Since the behavior of isolated animals deviates from normal primarily in emotional characteristics, and perhaps cognitively, alterations of the physiological response to emotion-producing experiences is suggested. Increased emotionality in these animals suggested that if altered, CORT levels would most likely be increased.

Finally, if isolation increased CORT levels, and impaired hippocampal function, it was suspected that handling might compensate for the negative effects of isolation.

Chapter 11: Experimental design and method

A. Subjects - subjects for the study were Long-Evans rats bred in the animal colony at the University of Arizona. The colony room is maintained on a 12/12 hour light/dark cycle with lights on at 10:00 pm. For purposes of breeding, 3 females and 2 males were placed together and left undisturbed for 4 days. Females were then housed individually until parturition.

The day of birth was delineated postnatal day 1 (PN1). On PN2, litters were culled to 8 pups, 4 male and 4 female, and each litter assigned to one of four groups.

1. Manipulation Procedure - In order to consider the effects, both separately and combined, of handling and isolation on later behavioral and structural parameters of development, the following groups were constituted:

Handling Only (HO) - this group received only the handling manipulation. On PN3 - PN13, inclusive, the dam was removed from the nest and placed in a separate cage. The litter was then removed and placed in a closed container approximately 6 inches by 4 inches. The pups remained in the container for 5 minutes, and were gently shaken for alternating 30 second periods until the 5 minutes had passed. Following the procedure, the pups and then the dam were returned to the home cage. Aside from this manipulation which occurred at 6:00 pm. on the designated days, the litter and the dam were

left undisturbed throughout the postnatal period until weaning on PN21.

Isolation Only (IO) - The IO group was exposed to the isolation manipulation as follows: On PN days 7, 9 and 11, the dam and then the pups were removed from the home cage. The pups were placed into individual compartments formed by dividing a 9 by 11 inch box into 8 equal sections. Approximately 4 inches of clean bedding was placed in the bottom of the box. The box was then elevated above a heating pad at a distance that allowed the "nests" to be maintained at 33 degrees C. The animals were removed from the dam and placed into the isolation nest at 9:00 am. on the scheduled isolation days, and remained there until 6:00 pm. Isolation time was thus 9 hours per session.

Handling Plus Isolation (HI) - Because it was suspected that isolation might produce an increased vulnerability to respond to the handling procedure with corticosterone elevations, a group receiving both the isolation and handling procedures was used. This group received exactly the same isolation procedure as the IO group, and exactly the same handling procedure as the HO group. The timing of the procedures was arranged in such a way that handling immediately followed isolation, with no return to the home nest between procedures.

Control (CO) - The control group remained undisturbed in the

maternity cage until weaning on PN21.

All litters were weaned on PN21 and housed in same sex, same treatment groups of 4 until behavioral testing began on PN60. All animals were briefly handled 2 times per week during the period PN21 - PN60 in order to prepare them for the behavioral test phase of the study.

B. Behavioral Testing

1. Cognitive Measure

Apparatus - A 6 ft. diameter fiberglass tank painted an off-white color was used for both the cue and place versions of the water maze. The tank was fitted with a stable platform that offered the animal escape from constant swimming, and which could be moved to any of the four quadrants of the tank.

Procedure - Animals were tested in groups of eight. The design was balanced in such a way that two animals from each treatment group (one of each sex) made up each test group of eight. Some of the animals (N=104) were tested on the place task, while the remaining animals (N=40) learned the cued version of the task.

For the place version of the water maze, the platform remained in one location throughout the two test days. The animals were given 8 training trials on each of the test days, followed on the second day by a single probe trial in which the platform was removed from the tank, and the

animals allowed to search for 60 seconds. During the training trials the animals were started from each of the four compass directions twice, with locations for each trial generated in a semi-random fashion.

In this task the animal must acquire and retain information about the position of the platform based on its location in the room. It has been shown that this task is sensitive to hippocampal function (Sutherland & Dyck, 1987; Morris, Garrud, Rawlins & O'Keefe, 1982). Latencies for the animal to locate the platform on each trial were recorded.

In the cued version of the water maze, the platform changed location on every trial and the animals' starting point remained stationary. The location of the platform was marked by a rubber ball hanging about 6 inches above the platform. As in the place task, each animal was given 8 trials per day for two consecutive days, followed by a probe trial at the end of the second day. The same measurement was collected as for the place task.

2. Emotional Measures

Each animal was placed into the center of the empty water tank, which had been marked into eight pie-shaped sections with cloth tape. The experimenter left the area, and the animal's behavior was recorded on video tape. On the first trial, the open wells provided a means for the animal to escape from the open area. The tapes were scored

by counting the number of lines the animal crossed and the latency to escape into one of the wells. Number of lines crossed was standardized to 60 seconds (latency for most animals) by multiplying the number of lines crossed by the fraction of 60 seconds the animal was observed. The second trial was given 24 hours after the first trial. Procedure was the same as for the first except that the wells were covered so that escape from the open area was not possible.

C. CORT Levels

This measure was obtained at the conclusion of behavioral testing. A small sample of the animals was used (N=32). The animals were brought into the lab from the animal room in groups of 8. After a wait of approximately 30 minutes, each animal was anesthetized with 30 mg./kg sodium nembutal injected intraperitonealy. A blood sample (0.5 ml.) was drawn from the tail vein, spun at 2500 RPM for 10 minutes, the plasma was removed and stored at -20 C. until assayed.

The assay was performed using a kit from ICN biomedical (Costa Mesa, CA). Essentially, CORT levels were determined by combining plasma samples with radioactive antibodies, and measuring the extent to which the hormone combined with these antibodies.

Chapter 12: Results

The data were first analyzed for each measure separately in order to determine whether any differences existed between the groups or between the sexes for each variable observed. The data were then subjected to a multivariate analysis in order to determine the relationships between the variables.

A. Activity

For the escape variable, the numbers of animals in each group that escaped were counted. About a third of the animals in the control, handled, and handling plus isolation groups left the arena before the trial ended. Fifty-nine percent of the isolated animals escaped (Table 1). A Chi-square analysis failed to find significant group differences. Forty-eight percent of the males escaped, as compared to 34% of the females. This difference was not significant.

The numbers of lines crossed on the first trial can be seen in Table 2. There were no significant group or sex differences in a 2-factor ANOVA.

On the second trial conducted the following day, an analysis of variance performed on the number of lines crossed indicated a group difference, $F(3, 122) = 6.44$, $p<.05$, a sex difference, $F(1,122) = 4.37$, $p<.05$, and a group by sex interaction $F(3,122) = 3.04$, $p<.05$. Handled

animals were more active than any of the other groups, and females were more active than males. The interaction was that females were more active than males in all groups except the HO group, where males were more active than females (Table 3).

B. Place task

There was a great deal of variability from one trial to the next for individual animals, thus four average latencies were computed for each animal. Each of these latencies consisted of an average for a 4-trial block. The data were then analyzed to provide answers to two questions: did animals differ in the speed with which the task was acquired, and did animals differ in overall performance level?

In order to answer the first question, slope scores were computed for each animal by subtracting one average latency from the preceding score: thus 3 slopes were tested. Additionally, the average for trials performed on the second day of testing was subtracted from the average for day 1, thus creating a fourth slope score. Two-factor ANOVA's were used to compare the scores. No group or sex differences were observed in any of the 4-trial slopes (Table 4). There was a difference between males and females for the slope from day 1 and day 2; $F(1,96)= 4.01$, $p<.05$. Males had a steeper slope (17.92) than females (13.54).

The second question: whether animals differed in overall performance, or the speed with which the task was learned, was analyzed by comparing the sum of latencies for all trials. A 2-factor hierarchical analysis of variance was used to compare the sum scores. There was a significant difference between the groups; $F(3,96)= 2.67$, $p<.05$. Protected F post-hoc tests revealed that both handled and isolated animals had higher sums than controls. There was not a significant sex difference in sum scores. There was an interaction however; $F(3,96)= 4.56$, $p<.01$. Males had lower combined latencies than females for all groups except for isolated animals, where females had lower latencies than males. This result is due to the fact that the males had longer latencies than control males; the isolated females performed very much like control females (Table 5).

For the probe trial, the number of time in seconds that each animal spent swimming in the quadrant that had been correct during training was analyzed. A 2-factor ANOVA was used to test differences between groups and sexes. The control animals spent more time swimming in the correct quadrant than any of the animals from other groups; $F(3,96)= 4.50$, $p<.01$. (Table 6). Protected F post-hoc tests showed that the handled and isolated animals differed from control rats.

There was also a sex difference: males spent more time

in the correct quadrant than females; $F(3,96)=5.0$, $p<.05$. There was no interaction between the variables.

C. Cue task

Forty animals performed the cue task. Data for this measure was analyzed as for the place task: a sum of latencies and slope scores between each of the four trial blocks were computed and a 2-factor, hierarchical analysis of variance computed. Sum scores did not differ between the groups or sexes (Table 7). No group differences were observed in any of the slope scores, however, there were sex differences. For the first slope, males had a steeper line than females; $F(1,32)= 6.24$, $p<.05$. Males also had a steeper slope on the second computed line; $F(1,32)= 6.38$, $p<.05$. There was no sex difference on slope three. (Table 8).

On the probe trial there were group and sex differences. Control animals spent more time swimming in the cued quadrant than any other group; $F(3,32)= 2.94$, $p<.05$. Protected F post hoc tests indicated that the handled and the handled plus isolated groups differed from control animals. There was also a sex difference: females spent more time in the correct quadrant than males; $F(1,32)= 3.96$, $p<.05$ (Table 9). There was no interaction between group and sex on the probe trial.

D. CORT Levels

CORT levels were analyzed with a two factor ANOVA. There was an effect of group, $F(3,24)= 3.09$, $p<.05$. Handled animals had lower CORT levels than controls, and isolated and handled + isolated animals had higher CORT levels than controls. There was a sex difference, $F(1,24)= 17.48$, $p<.05$. Females had higher CORT levels than males. There was also an interaction, $F(3,24)= 3.97$, $p<.05$. In the isolated group, males had lower CORT levels than controls, while isolated females had higher levels than control females (Table 10).

E. Handling VS Isolation

The data were separated in such a way as to examine the separate effects of handling and isolation. Data were put into 2x2 matrices with handling on one axis and isolation on the other (Tables 11-20). This allowed the comparison of all animals which had been handled, regardless of whether handling was combined with isolation, with animals not exposed to handling. The same comparison was made for isolated animals. A hierarchical analysis of variance was run for each variable. None of the variables related to activity, or to the cued version of the water task indicated significant differences due to handling or isolation. For the place version of the water maze, there was a significant effect of isolation: isolated animals spent less time than

nonisolated animals in the correct quadrant on the probe trial, $F(1,101)= 11.12$, $p<.05$. None of the other place task variables were significant.

F. Multivariate Analyses

Three analyses were run in order to test the relationships between the dependent variables. CORT levels were only available for a sample of the animals in the study, thus the test of the entire model had to be computed for only that sample. Two analyses were required to test the effect of activity on learning - this was due to the fact that each animal ran either the place or the cued version of the water tank task in order to eliminate the effects of previous testing on performance.

The correlation matrix for activity and place-learning variables can be seen in Table 21. The three activity variables correlated fairly well with each other. For the place learning variables, the sum of latencies for all trials correlated with the first slope, and with the probe trial, but other measures did not correlate highly. This analysis suggests that performance on the place task is not caused by differences in individual activity levels.

For the cue task also, activity measures failed to correlate with measures of performance. As for the place data, activity measures correlated well with each other, and the different learning measures were not reliably related to

other learning measures (Table 22).

The third analysis was a hierarchical linear model analysis used to determine the correlations of the variables (UniMult Statistical software was used. Gorsuch, 1991; Altadena, CA.). The model is depicted in figure 1; numbers represent the effect size coefficients for each variable in the model. The model tests the hypothesis that CORT levels affect activity levels, which in turn affect spatial learning. The variance has been partialed out of each variable in the order in which it appears in the model. The variables included in the model were chosen because of their ability to discriminate among the independent variables. Thus, the number of lines crossed on the second activity trial was used to represent activity, the sum of latencies for all trials was used for a measure of acquisition, and the amount of time in the correct quadrant on the probe trial was used for retention of the task. CORT levels had no effect on activity levels, or on the acquisition of the spatial water task, however, CORT was significantly related to retention of the task, $F(1,32)= 3.10$, $p<.05$. The correlation was negative indicating that increases in CORT predicted decreases in time in the correct quadrant. The activity measure was not related to retention or to acquisition.

The model was then run with the variable "sex"

controlled because the negative correlation of CORT to retention was inconsistent with the group differences indicated by the analyses computed for the individual tasks. There were reliable sex differences in the place measures, and in CORT levels, that could have produced a spurious correlation between the dependent variables in the model. Indeed, with sex controlled, the effect of CORT on the probe trial was no longer significant. However, the relation of CORT to sum was significant $F(1,32)= 3.07$. $p<.05$. This indicates that as CORT levels increased, sum decreased - the animals found the platform faster on the training trials.

Chapter 13: Discussion

These results indicate that handling and isolation are forms of early experience that affect the development of rats. Past experiments of handling infant rat pups have long been interpreted to mean that handling produced animals that were less emotional and better able to learn. The results presented here suggest a different interpretation. Because other studies have generally used only male animals, the results for males will be discussed first. The results of this study are consistent with previous findings: the handled males were less emotional. That is, they were less likely to engage in the fearful immobility in an open field that was observed in the control males. Second, the data from the cue task indicate that the handled males had no impairments on this task, although they did spend less time in the cued quadrant on the probe trial.

However, the place task produced a different result. Handled males did not solve the task as quickly as control males. Thus, control or "normal" male rats quickly learn to use spatial information to escape from the water, while they take somewhat longer to learn to use cues to guide their escape. This phenomenon has been taken as evidence of the primacy or dominance of place strategies for learning about environmental events (O'Keefe & Nadel, 1978). Normal rats trained to approach a goal in a specific location

demonstrate a preference for solving the problem by incorporating a map-like representation of the environment. When an animal is given a choice of solving the problem with this type of "place" strategy, or a strategy in which the animal learns to make a particular response (right or left turn) or approach a single signal-type cue, rats generally choose the place strategy. When solving a problem in which a cue or response solution is required, rats must suppress information about spatial location in order to accurately learn the cue response. Acquisition for the cue task was not impaired for the handled animals, but they did spend less time in the cued quadrant on the probe trial. One possible explanation for this result is that the behavior of the handled animals is controlled during acquisition and during the probe trial by a single stimulus: the cue. When the animal fails to find the platform after approaching the cue, it may leave the quadrant in order to reproduce the learned visual stimulus which controls its response. Thus a larger portion of the trial is spent outside of the cued quadrant. If, on the other hand, control animals suppress a dominant place strategy in order to learn the cue strategy, the cue may only be responsible for getting them to the vicinity where the platform has been found on past trials. Once in the quadrant, they may spend their time searching the local area for the platform - they do not rely as

strongly on the cue to determine their response.

These handled males had lower CORT levels than controls, and CORT was negatively correlated with acquisition latencies. This suggests that low levels of CORT produce slower learning. One possibility suggested by these results is that some minimum level of CORT is required for normal performance on a spatial task. While the reduced CORT levels in handled males may, as Meaney (1988) suggests, provide long-term protection from the accumulated damage of circulating CORT, in the short-term the results may be a decrease in CORT-mediated behaviors. For laboratory rats, a decrease in CORT levels and spatial ability during early life, with a concomitant reduction of cellular damage in later life may be a good thing. But for rats living in natural environments, these early life deficits may mean that the animal will fail to survive to old age.

The combined results of the activity and learning tasks for handled males suggest that these animals behave in ways similar to hippocampally lesioned rats. They engage in more activity in novel environments, although there is no evidence that this increased activity is associated with increased exploration. They are also impaired on the acquisition of a place task, without any corresponding deficits in general learning ability. Thus, handling of male rat pups appears to influence hippocampal function in a

negative way.

The cognitive effects of handling on male rats do not appear to extend to females. The females performed similarly to control females on measures of activity and both versions of the learning task.

The obvious next questions are: structurally, what is being affected by handling, and how does that structural difference translate to behavioral function? One explanation proposed by Meaney and associates is that handling affects concentrations of Type II receptors. Increased levels of these receptors results in enhanced negative feedback, and faster returns to baseline CORT levels. Lower CORT levels across long periods of time have the effect of protecting hippocampal cells from the cumulative damage that results from their presence. Thus, Meaney explains the behavioral change of handled animals as a result of altered (lowered) CORT levels, which produce a decrease in cell damage due to the presence of high CORT levels. Thus, Meaney's hypothesis explains the results of handling as a long-term protective mechanism which preserves numbers of cells. Enhanced behavior of aged handled animals is due to increased numbers of hippocampal cells. This theory says nothing of the immediate effects of altering CORT levels on behavioral function. The model analysis presented here suggests that increased CORT levels are

related to faster acquisition. Handled males, with decreased CORT levels were impaired on the speed of acquisition on the spatial task. Handled males took longer to learn the task, and did not remember it very well.

Thus, the behavioral effects of handling might be explained by alterations in the manner in which these animals respond to stressful situations - terminating the stress response while it is still needed for optimal responding to the environment means less effective responding.

The explanation for the effects of early isolation on later behavior is less clear. This manipulation is not well studied, and less is known of its behavioral, anatomical and physiological results. Two approaches to the interpretation of the data for isolated animals will be used. First, a comparison of control and isolated animals will help to determine how these animals are affected by the manipulation. Second, comparing the effects of isolation to those produced by handling will allow some consideration of common mechanisms for the results.

Isolated animals performed similarly to controls on the activity measure and the cued version of the water maze. They were impaired on the place task relative to controls, and this finding appears to be due to poor performance by the males. Thus, these animals do not have a behavioral

profile exactly like that of hippocampally lesioned animals, but rather a more selective deficit that is limited to spatial behavior. Perhaps more importantly, isolated animals had a reliable alteration of CORT levels: males had very low levels, while females had very high levels. Consistent with the model analysis, there were no activity differences associated with CORT changes, there was no difference on the slope scores, but the sum of latencies varied in a manner consistent with the CORT changes: females with high CORT levels did not differ from control females, but the lower CORT levels in males correlated with evidence of slow learning. Like the handled males, isolated males and females spent less time in the correct quadrant on the probe trial, although as mentioned above, this variable did not correlate with CORT levels.

In summary, handled only and isolated only animals (males) both performed poorly on a spatial task, but handled animals also had increased activity levels in an open field test. Neither group had deficits on a nonhippocampal learning task. If the explanation given for the effects of handling are correct, and if isolation also affects the stress response, it is difficult to imagine why activity levels are not also affected in the isolated animals. This difference in the two groups provides some reason to believe that the two manipulations exert their effects through

different mechanisms.

However, if handling and isolation interfere with development in different ways, then it would be expected that the combined effects of the manipulations would produce a cumulative deficit. This was not the case in this study. In fact the two manipulations appear to have cancelled each other out. The group exposed to combined handling and isolation performed similarly to controls on all measures tested, and as the handling vs. isolation tables and analyses demonstrate, when this combined group is examined, the only effect observed was a negative effect of isolation on the probe trial of the place task.

If handling increased Type II receptors, as Meaney's results suggest, handling may have the effect of lowering CORT levels in response to stressful situations and additionally of altering behavior performed under stressful conditions. Thus, there is a constellation of behavioral alterations, all of which may reflect a change from the adaptive patterns that normally accompany new or challenging events. If Type II receptors terminate the stress response, and reduce CORT, all of these behaviors may be altered. This explanation appears to explain the effects of handling quite well, but at the same time suggests that isolation effects either do not result from altering Type II receptors, or that in addition to altering Type II

receptors, isolation produces additional effects (Some plausible candidates include alterations of thyroid hormones or opiate receptors). Whatever the mechanism for these "additional" effects, it may be obliterated by handling.

Another possibility is that rather than affecting Type II receptors, isolation may alter levels of Type I receptors. Although little is presently known of the functions of these receptors, the fact that they behave differently in the hippocampus than in other brain and body locations suggests that they may selectively alter hippocampal function. The fact that they preferentially bind CORT in the hippocampus suggests that CORT levels in blood could influence the function of these receptors. Isolated animals had reduced CORT, but their behavior was not altered in a manner consistent with a change in response to stress in general as in handled animals, but in a more selective manner. Thus, Type II receptors may influence not only CORT levels, but all levels of the stress response system. Type I receptors on the other hand may influence a much more limited range of functions. If this hypothesis is true, it suggests that the mechanism of action affects males and females differently. It also suggests that high CORT levels do not interfere with hippocampal function, but that low levels negatively affect it.

The relationship between these 2 receptors and of the

interactions between receptors and structure is unknown at this time. Clearly, the functions of the hippocampal components of the stress response system need to be better understood. We must also understand the effects of handling and isolation as separate functions before any sense can be made of the interactions between these manipulations.

CORT Levels

The measurement of CORT levels in this study must be interpreted cautiously. There were few animals (8) sampled in each condition, and variability within groups was high. The direction of differences between groups and sexes was consistent with what is currently known. Females had higher CORT levels than males, and handled animals had lower levels than controls. Where the CORT data suggested an interesting difference was for the isolated animals. The isolated females had CORT levels that were higher than the range of normal (50 - 400 ng/ml). Although the elevated CORT levels did not transfer to any behavioral differences that were measured in this study, one characteristic of these animals was anecdotally noted. During the water tank test sessions isolated females became increasingly difficult to work with. This was due to what is probably best described as "agitated" behavior. These animals would race back and forth in their cages, vocalize when picked up and try to escape the grasp of the experimenter. This same sort of

behavior was noted by researchers studying effects of chronic CORT treatment on male rats (Stone, Egawa & McEwen, 1988). A systematic investigation of this behavior demonstrated that rats exposed to daily CORT injections engaged in more escape behavior - defined as attempts to pull away from someone holding the tail, and jumping off of an elevated platform. Decreased escape behavior was noted in adrenalectomized animals. Alterations of escape behavior were not correlated with increases in general motor activity (Stone, Egawa & McEwen, 1988).

In the present study, greatly elevated CORT levels produced a similar increase in escape behavior, but were not reliably related to the behavioral functions measured in any immediately obvious way. This suggests that high CORT levels do alter behavior, but do not necessarily affect hippocampal behaviors. Low CORT levels on the other hand were related to the ability to solve a spatial task. Both handled and isolated males had low CORT levels, and performed poorly on the place version of the water maze. Of course, this cannot be the whole explanation for differences in performance. Males have lower CORT levels than females, and yet they consistently outperform females on the spatial task used in this study. One interpretation that is suggested by these results is that some minimum level of CORT is required for accurate spatial performance, but that

performance is not altered by increases past this point.

An additional factor must also be considered. Levels of CORT circulating in the blood do not completely determine the effects of this hormone. Like all biochemical substances in the body, CORT requires receptors in order to produce its effects. It is known that glucocorticoid receptors (at least Type II) have the capacity to autoregulate - that is their numbers increase and decrease in response to levels of the hormone in the blood. It is also known that experience early in life can lead to apparently permanent alterations of glucocorticoid receptor concentrations (Meaney, 1988, 1991). In the study reported above (Stone, et al., 1988), increases in escape behavior were not observed before the second day of CORT administration. This suggests that it requires some period of time before high CORT levels can influence the Type II receptors. One way to think of these receptors may be as slow-acting regulators of internal homeostatic processes that can be influenced by environmental conditions. Thus, animals with higher or lower levels of these receptors would have altered CORT levels as a result of the feedback action of Type II receptors. The manner in which CORT exerts immediate influences on behavior may be produced by a faster-acting interaction with receptors designed to respond to changes in current conditions of the environment. As

mentioned in the introduction, some researchers believe that the Type I receptors may be responsible for the relationship between arousing situations and learning that occurs in those situations.

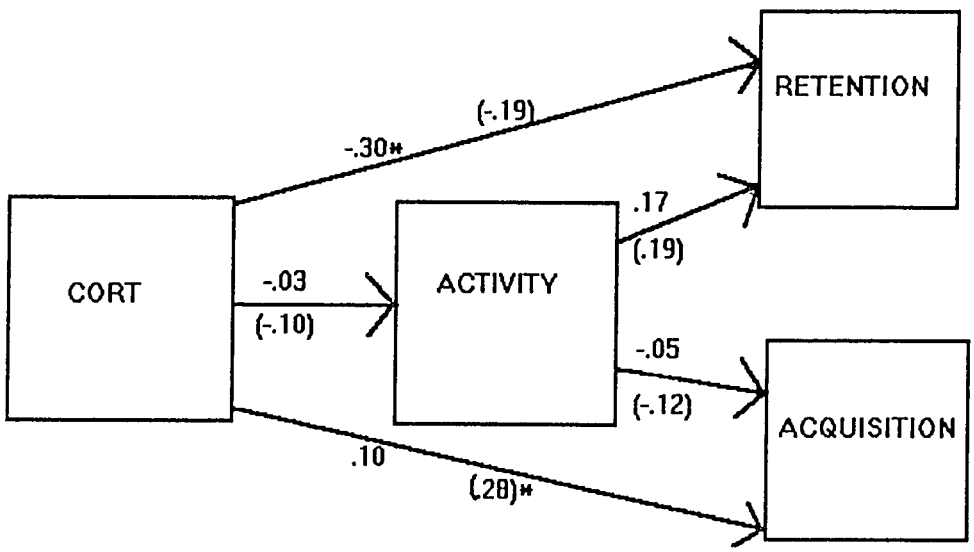
Chapter 14: Conclusions

Two important conclusions can be drawn from the experiments presented here. First, the handling of infant rat pups is not necessarily a beneficial experience for animals that must survive in natural conditions. While nonhippocampal learning tasks may be enhanced when the influence of the hippocampus is lessened or removed as a result of handling, the behaviors mediated by the hippocampus are essential to an animal's ability to survive in the wild. Likewise, the protection of the hippocampus from the damage incurred by a lifetime of stress-induced elevations of glucocorticoids, is of little value unless the animal survives to old age. The cost for survival during early life may be a certain amount of damage to some neural (and non-neural) structures.

The second finding is that isolation of infant rats from their mother alters circulating levels of corticosterone later in life. Males have lower levels than normal, and have deficits in hippocampally mediated behavior. Females have higher levels than controls and do not exhibit any hippocampal deficits. This finding suggests that some minimal level of CORT may be necessary for animals to engage in the sorts of adaptive behaviors known to be mediated by the hippocampus.

Figure 1

Linear model analysis for all dependent variables.**



* p<.05
** Numbers on directional arrows are effect size measures. Effect sizes with sex differences partialled out are indicated in parentheses.

Table 1

Percentage of animals that escaped from an open field*

		SEX		
		Male	Female	
GROUP	Control	22%	7%	16%
	Handled Only	45%	24%	31%
	Isolated Only	53%	53%	53%
	Handled& Isolated	37%	19%	28%
		38%	26%	32%

*N=128

Table 2

Activity - Mean number of lines crossed on trial 1*

		SEX		
		Male	Female	
GROUP	Control	4.4 sd=3.8	8.1 sd=4.7	6.0 sd=4.6
	Handled Only	10.4 sd=7.0	8.3 sd=5.2	9.0 sd=5.9
	Isolated Only	6.9 sd=5.3	9.0 sd=6.5	8.0 sd=6.0
	Handled& Isolated	5.3 sd=5.0	8.2 sd=5.4	6.7 sd=5.4
		6.4 sd=5.6	8.4 sd=5.5	7.5 sd=5.6

*N=128

Table 3

Activity - Mean number of lines crossed on trial 2*

		SEX		
		Male	Female	
GROUP	Control	4.7 sd=4.9	8.7 sd=4.8	6.4 sd=5.3
	Handled Only	13.7 sd=6.6	10.1 sd=4.4	11.4 sd=5.5
	Isolated Only	6.8 sd=5.6	8.5 sd=4.6	7.7 sd=5.2
	Handled& Isolated	4.6 sd=3.4	9.6 sd=5.4	7.1 sd=5.1
		6.8 sd=6.1	9.3 sd=4.9	8.2 sd=5.6

*N=128

Table 4

Place task - Average slopes for groups and sexes*

		SEX		
		Male	Female	
GROUP	Control	S1= 25.3 S2= -2.0 S3= 10.8 S4= 16.1	S1= 17.1 S2= 3.8 S3= 13.9 S4= 19.1	S1= 21.8 S2= 0.5 S3= 12.1 S4= 17.4
	Handled Only	S1= 10.4 S2= 10.4 S3= 10.4 S4= 20.8	S1= 11.3 S2= -3.9 S3= 16.7 S4= 10.1	S1= 10.9 S2= 1.4 S3= 14.4 S4= 14.0
	Isolated Only	S1= 18.9 S2= 1.6 S3= 15.9 S4= 19.0	S1= 21.2 S2= -0.3 S3= 9.8 S4= 15.2	S1= 20.3 S2= 0.5 S3= 12.2 S4= 16.7
	Handled& Isolated	S1= 18.3 S2= 4.2 S3= 7.7 S4= 17.3	S1= 20.3 S2= -4.0 S3= 8.5 S4= 10.4	S1= 19.3 S2= 0.1 S3= 8.1 S4= 13.9
		S1= 19.1 S2= 2.9 S3= 11.0 S4= 18.0	S1= 17.1 S2= -1.3 S3= 12.5 S4= 13.5	S1= 18.0 S2= 0.6 S3= 11.8 S4= 15.6

*Each slope (S) represents the difference between one 4-trial block and the following block. N=104.

Table 5

Place task - Average sum scores for groups and sexes*

		SEX		
		Male	Female	
GROUP	Control	399 sd= 103	502 sd=103	443 sd= 115
	Handled Only	453 sd= 146	591 sd= 154	540 sd= 165
	Isolated Only	591 sd= 133	470 sd= 129	519 sd= 143
	Handled& Isolated	484 sd= 134	480 sd= 134	482 sd= 134
		472 sd= 145	516 sd= 143	495 sd= 145

*Numbers represent combined total latencies for all trials. N=104.

Table 6

Place task - Percentage of time spent in correct quadrant on probe trial*

		SEX		
		Male	Female	
GROUP	Control	52 sd= 9.2	40 sd= 6.2	47 sd= 8.8
	Handled Only	47 sd= 9.7	40 sd= 5.4	42 sd= 7.6
	Isolated Only	40 sd= 6.2	34 sd= 7.7	36 7.4
	Handled& Isolated	34 sd= 7.4	37 sd= 6.7	36 sd= 7.2
		44 sd= 9.4	38 sd= 6.7	41 sd= 8.3

*N=104

Table 7

Cue task - Average slope scores for groups and sexes*

		SEX		
		Male	Female	
GROUP	Control	S1= 22.8 S2= 7.3 S3= 9.9 S4= 23.7	S1= 16.9 S2= 0.9 S3= 8.2 S4= 13.5	S1= 19.9 S2= 4.1 S3= 9.1 S4= 18.6
	Handled Only	S1= 16.7 S2= 7.0 S3= 4.4 S4= 17.5	S1= 24.8 S2= -6.4 S3= 17.9 S4= 14.9	S1= 20.8 S2= 0.3 S3= 11.2 S4= 16.2
	Isolated Only	S1= 15.6 S2= 3.0 S3= 3.8 S4= 12.7	S1= 20.4 S2= -0.4 S3= 8.3 S4= 14.0	S1= 18.0 S2= 1.3 S3= 6.0 S4= 13.4
	Handled& Isolated	S1= 30.9 S2= -0.5 S3= 8.1 S4= 19.0	S1= 20.7 S2= -5.8 S3= 11.1 S4= 10.1	S1= 25.8 S2= -3.2 S3= 9.6 S4= 14.5
		S1= 21.5 S2= 4.2 S3= 6.6 S4= 18.2	S1= 20.7 S2= -2.9 S3= 11.4 S4= 13.1	S1= 21.1 S2= 0.6 S3= 9.0 S4= 15.7

*Each slope (S) represents the difference from one 4-trial block to the next. N=40.

Table 8

Cue task - Average sum scores for groups and sexes*

		SEX		
		Male	Female	
GROUP	Control	428 sd= 88.5	345 sd= 86.0	387 sd= 96.6
	Handled Only	388 sd= 94.1	404 sd= 76.8	396 sd= 86.3
	Isolated Only	341 sd= 64.2	378 sd= 64.7	360 sd= 67.1
	Handled& Isolated	392 sd= 43.1	419 sd= 91.3	405 sd= 72.6
		387 sd= 81.4	387 sd= 85.0	387 sd= 83.2

*N=40

Table 9

Cue task - Percentage of time spent in correct quadrant on probe trial*

		SEX		
		Male	Female	
GROUP	Control	52 sd= 3.7	70 sd= 8.6	62 sd= 8.8
	Handled Only	50 sd= 8.9	48 sd= 3.4	49 sd= 6.7
	Isolated Only	47 sd= 5.6	59 sd= 9.9	53 sd= 8.8
	Handled& Isolated	44 sd= 4.8	49 sd= 2.1	46 sd= 3.9
		48 sd= 6.4	57 sd= 8.8	52 sd=8.1

*N=40

Table 10

Corticosterone levels for groups and sexes*

		SEX		
		Male	Female	
GROUP	Control	154.5 sd= 52.3	205.7 sd= 100.2	180.1 sd= 83.9
	Handled Only	122.0 sd= 40.2	168.3 sd= 103.7	149.8 sd= 87.2
	Isolated Only	105.7 sd= 84.5	446.2 sd= 60.9	276.0 sd= 185.5
	Handled& Isolated	166.0 sd= 55.3	254.2 sd= 127.6	215.0 sd= 111.0
		137.1 sd= 65.0	257.3 sd= 145.4	202.3 sd= 130.4

*Numbers represent ng./ml. corticosterone in blood. N=32.

Table 11

Handling VS. Isolation: Percentage of animals that escaped from an open field*

		Isolated		
		YES	NO	
Handled	YES	29%	30%	30%
	NO	53%	16%	34%
		41%	23%	32%

*Observed on the first day of trials for the activity measure. N = 128.

Table 12

Handling VS. Isolation: Number of lines crossed on trial 1 of the activity measure*

		Isolated		
		YES	NO	
Handled	YES	6.8	8.9	7.9
	NO	8.0	6.0	7.0
		7.4	7.5	7.4

*N=128.

Table 13

Handling VS. Isolation: Number of lines crossed on trial 2 of the activity measure*

		Isolated		
		YES	NO	
Handled	YES	6.9	11.4	9.2
	NO	7.7	6.4	7.1
		7.3	9.0	8.2

*N=128.

Table 14

Handling VS. Isolation: Place task - Slope from first to second day of training*

		Isolated		
		YES	NO	
Handled	YES	13.9	13.9	13.9
	NO	17.6	17.4	17.5
		15.7	15.8	15.6

*N=104

Table 15

Handling VS. Isolation: Sum scores for the place task*

		Isolated		
		YES	NO	
Handled	YES	482	570	526
	NO	516	443	477
		499	502	495

*N=104

Table 16

Handling VS. Isolation: Seconds spent in correct quadrant on the probe trial for the place task*

		Isolated		
		YES	NO	
Handled	YES	21	24	23
	NO	23	28	26
		22	26	24

*Total trial time was 60 seconds. N=104

Table 17

Handling VS. Isolation: Scores for day 1 to day 2 on the cue task*

		Isolated		
		YES	NO	
Handled	YES	18.6	13.4	16.0
	NO	16.2	14.5	15.4
		17.4	13.9	15.7

*N=40

Table 18

Handling VS. Isolation: Sum of latencies for all trials - cue task.

		Isolated		
		YES	NO	
Handled	YES	387	360	373
	NO	396	405	401
		391	383	387

*N=40

Table 19

Handling VS. Isolation: Amount of time spent in correct quadrant on the probe trial of the cue task*

		Isolated		
		YES	NO	
Handled	YES	27	30	29
	NO	32	37	34
		30	33	31

*Maximum time possible was 60 seconds. N=40.

Table 20

Handling VS. Isolation: Blood levels of corticosterone. Measured as ng./ml.*

	Isolated			
Handled		YES	NO	
	YES	140	138	139
	NO	250	100	175
		195	117	157

*N=32

Table 21

Correlations between activity measures and place learning measures.*

	1	2	3	4	5	6	7	8	9
1	1.0								
2	.43	1.0							
3	.36	.21	1.0						
4	-.10	-.03	.01	1.0					
5	-.13	-.05	-.04	-.33	1.0				
6	.19	-.09	.01	-.44	.20	1.0			
7	-.08	-.06	.01	.06	-.13	-.57	1.0		
8	.06	.05	.04	.19	.10	.06	-.40	1.0	
9	.06	-.14	.05	-.13	.03	-.02	.74	.03	1.0

VARIABLE LIST:
1. Lines crossed on trial #1
2. Lines crossed on trial #2
3. Latency to escape open field
4. Sum of latencies for all training trials
5. Time spent in correct quadrant on probe trial
6. Slope of line from first 4 to second 4 trials
7. Slope of line from second 4 to third 4 trials
8. Slope of line from third 4 to fourth 4 trials
9. Slope of line from day 1 to day 2 trials

* N=104

Table 22

Correlations between activity measures and cue learning measures.*

	1	2	3	4	5	6	7	8	9
1	1.0								
2	.74	1.0							
3	.37	.21	1.0						
4	-.02	-.02	-.23	1.0					
5	.08	-.09	.08	-.24	1.0				
6	.07	.03	-.21	.19	-.17	1.0			
7	-.03	-.03	-.04	-.32	.09	-.76	1.0		
8	.01	-.10	-.00	.61	-.18	.09	-.76	1.0	
9	.02	-.07	-.20	.10	-.12	.55	.63	-.27	1.0

VARIABLE LIST:
1. Lines crossed on trial #1
2. Lines crossed on trial #2
3. Latency to escape open field
4. Sum of latencies for all training trials
5. Time spent in correct quadrant on probe trial
6. Slope of line from first 4 to second 4 trials
7. Slope of line from second 4 to third 4 trials
8. Slope of line from third 4 to fourth 4 trials
9. Slope of line from day 1 to day 2 trials

* N=40

THE UNIVERSITY OF ARIZONA
TUCSON, ARIZONA 85721
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

FINAL

VERIFICATION OF REVIEW
BY THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

PHS Assurance No. A-3248-01 - USDA No. 86-3

IACUC Control # 89-0022

Title: Early Experience: Learning and Glucocorticoids

Principal Investigator: Lynn Nadel

Department: Psychology

Submission Date: January 30, 1989

Agency: NSF

The University of Arizona Institutional Animal Care and Use Committee reviews all sections of proposals relating to animal care and use. The above-named proposal:

[ ] Has been reviewed and approval withheld.

[XX] Has been reviewed and approved by IACUC on February 10, 1989
Revisions (if any), are listed below:
NONE

Approval valid through February 9, 1992

Michael A. Cusanovich, Ph.D.
Vice President for Research

Date: February 2, 1990

REFERENCES

Aggleton, J.P., Blindt, H.S. & Rawlins, J.N.P. (1989) Effects of amygdaloid and amygdaloid-hippocampal lesions on object recognition and spatial working memory in rats. *Behavioral Neuroscience, 103,* 962-974.

Angevine, J.A., Jr. (1965) Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Experimental Neurology, Supplement 2,* 1-70.

Angevine, J.A. & Cotman, C. (1981) *Principles of neuroanatomy.* Oxford University Press, New York.

Altman, J., Das, G.D. & Anderson, W.J. (1968) Effects of infantile handling on morphological development of the rat brain: an exploratory study. *Developmental Psychobiology, 1,* 10-20.

Barnes, C.A., Nadel, L. & Honig, W.K. (1980) Spatial memory deficit in senescent rats. *Canadian Journal of Psychology, 34,* 29-39.

Baudry, M., Arst, D., Oliver, M. & Lynch, G. (1981) Development of glutamate binding sites and their regulation by calcium in rat hippocampus. *Developmental Brain Research, 1,* 37-48.

Bayer, S.A. (1985) Hippocampal region. In G. Paxinos (Ed.) *The Rat Nervous System, Volume 1, Forebrain and Midbrain.* Academic Press, Australia.

Beatty, P.A., Beatty, W.W., Bowman, R.E. & Gilchrist, J.C. (1970) The effects of ACTH, adrenalectomy and dexamethasone on the acquisition of an avoidance response in rats. *Physiology and Behavior, 5,* 939-944.

Becker, J. Walker, J. & Olton, D. (1980) Neuroanatomical bases of spatial memory. *Brain Research, 200,* 307-320.

Bohn, M. (1984) Glucocorticoid induced teratologies of the nervous system. In: J. Yanai (Ed.) *Neurobehavioral Teratology.* Elsevier Science Publishers, BV.

Bohus, B. (1973) Pituitary-adrenal influences on avoidance and approach behavior of the rat. *Progress in Brain Research, 39,* 407-420.

Borrell, J. de Kloet, E.R. & Bohus, B. (1984) Corticosterone decreases the efficacy of adrenaline to affect passive avoidance retention of adrenalectomized rats. *Life Sciences, 34,* 99-105.

Buck, R. (1984) *The communication of emotion.* Guilford, New York.

Chevalier, J.A. & Levine, S. (1955) The effects of shock and handling in infancy on adult avoidance learning. *The American Psychologist, 10,* 432.

Chozik, B.S. (1983) The behavioral effects of lesions of the hippocampus: a review. *International Journal of Neuroscience, 22,* 63-80.

Cohen, N.J., Eichenbaum, H., Deacedo, B.S. & Corkin, S. (1985) Different memory systems underlying acquisition of procedural and declarative knowledge. *Annals of the New York Academy of Sciences, 444,* 54-71.

Crain, B., Cotman, C., Taylor, D. & Lynch, G. (1973) A quantitative electron microscopic study of synaptogenesis in the dentate gyrus of the rat. *Brain Research, 63,* 195-204.

Creery, B.L. & Bland, B.H. (1980) Ontogeny of fascia dentata electrical activity and motor behavior in the Dutch Belted rabbit. *Experimental Neurology, 67,* 554-572.

Daly, M. (1973) Early stimulation of rodents: a critical review of present interpretations. *British Journal of Psychology, 64 (3),* 435-460.

Darwin, C. (1872) The expressions of emotions in man and animals. In: B.J. Loewwnberg (Ed.) *Charles Darwin: evolution and natural selection.* Beacon Press, Boston.

De Kloet, E.R., Ratka, A., Reul, J.H.M., Sutanto, W. & Van Eekelen, J.A.M. (1987) Corticosteroid receptor types in brain: regulation and putative function. *Annals of the New York Academy of Science, 512,* 351-361.

De Kloet, E.R., Veldhuis, D. & Bohus, B. (1980) Significance of neuropeptides in the control of corticosterone receptor activity in rat brain. In: G. Pepeu, M.J. Kuhar & S.J. Enna (Eds.) *Receptors for neurotransmitters and peptide hormones*. Raven Press, NY.

Denenberg, V.H. (1967) Stimulation in infancy, emotional reactivity, and exploratory behavior. In: D.C. Glass (Ed.) Biology and Behavior: *Neurophysiology and Emotion*. New York. Rockefeller University Press and Russell Sage Foundation.

Denenberg, V.H. & Morton, J.R.C. (1962) Effects of environmental complexity and social groupings upon modification of emotional behavior. *Journal of Comparative and Physiological Psychology, 55 (2)*, 242-246.

Denenberg, V.H., Rosenberg, K.M., Haltmeyer, G.C. & Whimby, A.E. (1969) Programming life histories: effects of stress in ontogeny upon emotional reactivity. *Merrill-Palmer Quarterly*, 109-116.

de Weid, D. (1964) Influence of anterior pituitary on avoidance learning and escape behavior. *American Journal of Physiology, 207,* 255-59.

de Wied, D., Bohus, B., van Ree, J.M. & Urban, I. (1978) Behavioral and electrophysiological effects of peptides related to lipotropin. *Journal of Pharmacology and Experimental Therapy, 204,* 570-580.

Dollard, J., Doob, L., Miller, N., Mower, O. & Sears, R. (1939) *Frustration and aggression.* Yale University Press, New Haven, CT.

Dunn, A.J. (1980) Neurochemistry of learning and memory: an evaluation of recent data. *Annual Review of Psychology, 31,* 343-390.

DuPreez, P.D. (1964) The persistence of some effects of handling in infancy on the behavior of the adult rat. *Quarterly Journal of Experimental Psychology, 16,* 147-155.

Elmadjian, F. (1959) Excretion and metabolism of epinephrine. *Pharmacological Review, 11,* 409-415.

Eslinger, P.J. & Damasio, A.R. (1986) Preserved motor learning in Alzheimer's disease: implications for anatomy and behavior. *Journal of Neuroscience, 6,* 3006-3009.

Fass, B. (1983) Temporal changes in open-field activity following progressive lesions of entorhinal cortex: evidence for enhanced recovery. *Behavioral and Neural Biology, 37,* 108-124.

Feigley, D.A., Parsons, P.J., Hamilton, L.W. & Spear, N.E. (1972) Development of habituation to novel environments in the rat. *Journal of Comparative and Physiological Psychology, 79,* 443-452.

Feldman, S. & Conforti, N. (1980) Participation of the dorsal hippocampus in the glucocorticoid feedback effect on adrenocortical activity. *Neuroendocrinology, 30,* 52-55.

File, S.E. (1978) The ontogeny of exploration in the rat, habituation and effects of handling. *Developmental Psychobiology, 11,* 321-328.

Foster, T.C., Castro,C.A. & McNaughton, B.L. (1989) Spatial selectivity of hippocampal neurons: dependence on preparedness for movement. *Science, 244,* 1580-1582.

Glickman, S.E., Higgins, T.J. & Isaacson, R.L. (1970) Some effects of hippocampal lesions on the behavior of mongolian gerbils. *Physiology and Behavior, 5,* 931-938.

Gold, P.E. & Delanoy,R.L. (1981) ACTH modulation of memory storage processing. In: J.L. Martinez, R.A. Jensen, R.B. Messing, H. Rigter & J.L. McGaugh (Eds.) *Endogenous peptides in learning and memory processes.* Academic Press, NY.

Goodall, M (1962) Sympathoadrenal response to gravitational stress. *Journal of Clinical Investigation, 41,* 197-202.

Guth, S., Levine, S. & Seward, J.P. (1971) Appetitive acquisition and extinction effects with exogenous ACTH. *Physiology and Behavior, 7,* 195-200.

Harlow, H.F. & Zimmermann (1959) Affectional responses in the infant monkey. *Science, 130,* 421-432.

Harlow, H.F., Harlow, M.K., Schiltz, K.A. & Mohr, D.J. (1971) The effect of early adverse and enriched environments on the learning ability of Rhesus monkeys. In: L.E. Jarrard (Ed.) *Cognitive processes in nonhuman primates.* Academic Press. New York.

Hebb, D.O. (1955) Drives and the CNS (conceptual nervous system). *Psychological Review, 62,* 243-254.

Hinde & Spencer-Booth (1971) Effects of brief separation from mother on Rhesus monkeys. *Science, 17,* (3992), 111-118.

Izard, C.E. (1989) The structures and functions of emotions: Implications for cognition, motivation and personality. In: I.S. Cohen (Ed.) *The G. Stanley Hall lecture series, 9,* 39-73. American Psychological Association, Washington, D.C.

Jarrard, L., Isaacson, R.L. & Wickelgren, W.O. (1964) Effects of hippocampal ablation and intertrial interval on runway acquisition and extinction. *Journal of Comparative and Physiological Psychology, 57,* 442-444.

Jarrard, L.E., O'Kaich, H., Steward, O. & Goldschmidt, R.B. (1984) On the role of hippocampal connections in the performance of place and cue tasks: comparisons with damage to hippocampus. *Behavioral Neuroscience, 98,* 946-954.

Jefferies, D., Copolov, D., Irby, D. & Funder, J. (1983) Behavioral effect of adrenalectomy: reversal by glucocorticoids or [D-alpha^2, MET5] enkephalinamide. *European Journal of Pharmacology, 92,* 99-103.

Keller-Wood, M. & Dallman, M. (1984) Corticosteroid inhibition of ACTH secretion. *Endocrinology Reviews, 5,* 1-24.

Kesner, R.P. (1990) Learning and memory in rats with an emphasis on the role of the hippocampal formation. In: R.P. Kesner & D.S. Olton (Eds.) *Neurobiology of comparative cognition.* Earlbaum, Hillsdale, NJ.

Krieger, D.T. & Liotta, A.S. (1979) Pituitary hormones in brain: where, how, and why? *Science, 205,* 366-372.

Kurz, E.M., Harkins, B.L. & Nadel, L. (1984) Ontogeny and plasticity of exploratory behavior and hippocampal development in rats. Poster presented at Second Conference on the Neurobiology of Learning and Memory, University of California, Irvine.

LeBlanc, M.O. and Bland, B.H. (1979) Developmental aspects of hippocampal electrical activity and motor behavior in the rat. *Experimental Neurology, 66,* 220-237.

Levine, S. (1959) The effects of differential infantile stimulation on emotionality at weaning. *Canadian Journal of Psychology, 13 (4),* 243-247.

Levine S. & Lewis, G.W. (1959) Critical period for effects of infantile experience on maturation of stress response. *Science, 129,* 42-43.

Levine, S. (1962) The effects of infantile experience on adult behavior. In: A.J. Bachrach (Ed.), *Experimental Foundations of Clinical Psychology.* New York: Basic Books.

Maier, S.E., Vanderhoff, P., & Crowne, D.P. (1988) Multivariate analysis of putative measures of activity, exploration, emotionality, and spatial behavior in the hooded rat (Rattus Norvegicus). *Journal of Comparative Psychology, 102,* 378-387.

McEwen, B.S. (1987) Steroid hormones and the brain: linking 'nature' and 'nurture'. *Neurochemical Research, 38,* 45-60.

McEwen, B.S. & Brinton, R.E. (1987) Neuroendocrine aspects of adaptation. In: E.R. de Kloet, V.M. Weigant & D. de Wied, (Eds.) *Progress in Brain Research, 72,* 11-26.

McGaugh, J.L. (1983) Hormonal influences on memory. *Annual Review of Psychology, 34,* 297-323.

McGeer, P.L. & McGeer, E.G. (1980) Chemistry of mood and emotion. *Annual Review of Psychology, 31,* 273-307.

Meaney, M.J., Aitken, D.H., Bodnoff, S.R., Iny, L.J. & Sapolsky, R.M. (1985) The effects of postnatal handling on the development of the glucocorticoid receptor systems and stress recovery in the rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 9,* 731-734.

Meaney, M.J., Aitken, D.H., Bodnoff, S.R., Iny, L.J., Tatarewicz,J.E., Sapolsky, R.M. (1985) Early postnatal handling alters glucocorticoid receptor concentrations in selected brain regions. *Behavioral Neuroscience, 990 (1),* 765-770.

Meaney, M.J., Aitken, D.H., van Berkel, C., Bhatnagar, S. & Sapolsky, R. (1988) Effects of neonatal handling on age-related impairments associated with the hippocampus. *Science, 239,* 766-768.

Meaney, M.J., Aitken, D.H., Viau, V., Sharma, S. & Sarrieau, A. (1989) Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal Type II glucocorticoid receptor binding in the rat. *Neuroendocrinology, 50,* 597-604.

Meaney, M.J., Aitken, D.H., Bhatnagar, S. & Sapolsky, R. (1991) Postnatal handling attenuates certain neuroendocrine, anatomical, and cognitive dysfunctions associated with aging in female rats. *Neurobiology of Aging, 12,* 31-38.

Melzack, R. & Thompson, W.R. (1956) Effects of early experience on social behavior. *Canadian Journal of Psychology, 10,* 82-90.

Micco, D.J., McEwen, B.S. & Shein, W. (1979) Modulation of behavioral inhibition in appetitive extinction following manipulation of adrenal steroids in rats: implications for involvement of the hippocampus. *Journal of Comparative and Physiological Psychology, 93,* 323-329.

Micco, D.J. & McEwen, B.S. (1980) Glucocorticoids, the hippocampus and behavior: interactive relation between task activation and steroid binding specificity. *Journal of Comparative and Physiological Psychology, 94,* 624-633.

Milner, B. (1966) Amnesia following operation on the temporal lobes. In: C.W.M. Whitty & O.L. Zangwill (Eds.) *Amnesia*. Butterworths, 109-133.

Mitchell, D.E. (1980) The influence of early visual experience on visual perception. In: C.S. Harris (Ed.) *Visual coding and adaptability*, Earlbaum, Hillsdale, NJ.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P. & O'Keefe, J. (1982) Place navigation in rats with hippocampal lesions. *Nature, 297,* 681-683.

Muller, R.U. & Kubie, J.L. (1989) The firing of hippocampal place cells predicts the future position of freely moving rats. *Journal of Neuroscience, 9,* 4101-4110.

Nadel, L. & Willner, J. (1990) Some implications of postnatal maturation of the hippocampus. In: V. Chan-Palay & C. Kohler (Eds.) *The Hippocampus: New Vistas*. A.R. Liss. New York.

Nauta, W.J.H. & Feirtag, M. (1991) The organization of the brain. In: R. Llinas (Ed.). *The workings of the brain: Development, memory and perception*. W.H. Freeman & Co., New York.

O'Keefe, J. & Nadel, L. (1978) *The hippocampus as a cognitive map.* The Clarendon Press, Oxford.

O'Keefe, J., Nadel, L., Keightly, S. & Kill, D. (1975) Fornix lesions selectively abolish place learning in the rat. *Experimental Neurology, 48,* 152-166.

Olton, D.S. & Papas, D.C. (1979) Spatial memory and hippocampal function. *Neuropsychologica, 17,* 689-672.

Papez, J.W. (1937) A proposed mechanism of emotion. *Archives of Neurology and Psychiatry, 38,* 725-743.

Pfister, H.P. (1979) The glucocorticosterone response to novelty as a psychological stressor. *Physiology & Behavior,* 23, 649-652.

Plutchik, R. (1985) On emotion: the chicken-and-egg problem revisited. *Motivation and Emotion, 9,* 197-200.

Rasmussen, M., Barnes, C. & McNaughton, B. (1989) A systematic test of configural mapping, working memory and temporal discontiguity theories of hippocampal function. *Psychobiology, 24,* 24-35.

Renner, M.J. (1987) Neglected aspects of exploratory and investigatory behavior. *Psychobiology, 18,* 16-22.

Reul, J.M.H. & DeKloet, E.R. (1985) Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology, 117,* 2505-2511.

Rosenfeld, P., Sutanto, W., Levine, S. & DeKloet, E.R. (1988) Ontogeny of Type I and Type II corticosteroid receptors in the rat hippocampus. *Developmental Brain Research, 42,* 113-118.

Rosenfeld, P., Van Eekelen, J.A.M., Levine, S. & De Kloet, E.R. (1988) Ontogeny of the Type 2 glucocorticoid receptor in discrete rat brain regions: an immunocytochemical study *Developmental Brain Research, 42,* 119-127.

Rudy, J.W., Stadler-Morris, S. & Albert, P. (1987) Ontogeny of spatial navigation behaviors in the rat: Dissociation of "proximal"- and "distal" - cue based behaviors. *Behavioral Neuroscience, 101,* 62-73.

Sakurai, Y. (1990) Cells in the rat auditory system have sensory-delay correlates during the performance of an auditory working memory task. *Behavioral Neuroscience, 104,* 856-868.

Santucci, A.C., Riccio, D.C. & Treichler, F.R. (1989) Role of ACTH in recovery from retrograde amnesia induced by hypothermia in rats. *Behavioral Neuroscience, 103,* 1267-1275.

Sapolsky, R., Armanini, M., Sutton, S. & Plotsky, P. (1989) Elevation of hypophysial portal concentrations of adrenocorticotrophin secretagogues after fornix transection. *Endocrinology, 125,* 2882-2887.

Sapolsky, R., Krey, L. & McEwen, B. (1984) Glucocorticoid sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. *Proceedings of the National Academy of Science, USA., 81,* 6174-6177.

Sapolsky, R.M., McEwen, B.S. & Rainbow, T.C. (1983) Quantitative autoradiography of [3H]corticosterone receptors in rat brain. *Brain Research, 271,* 331-336.

Sapolsky, R.M. & Meaney, M.J. (1986) Maturation of the adrenocortical stress response: Neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Research Reviews, 11,* 65-76.

Sarrieau, A., Sharma, S. & Meaney, M.J. (1988) Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors. *Developmental Brain Research, 43,* 158-162.

Schenk, F., Inglin, F. & Gyger, M. (1983) Activity and exploratory behavior after lesions to the medial entorhinal cortex in the woodmouse (*Apodemus sylvaticus*). *Behavioral and Neural Biology, 37,* 89-107.

Schlessinger, A.R., Cowan, W.M. & Gottlieb, D.I. (1973) An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *Journal of Comparative Neurology, 159,* 149-176.

Smith, A.M. (1967) Infantile stimulation and the Yerkes-Dodson law. *Canadian Journal of Psychology, 1,* 285-293.

Somerville, E. (1979) Postnatal development of the hippocampus in the rat (Rattus norvegicus), correlations between hippocampal anatomy, electroencephalogram and behavior. Unpublished Ph.D. thesis, University College, London.

Speakman, A. & O'Keefe, J. (1989) Hippocampal place cells and hippocampal theories. In: V. Chan-Palay & C. Kohler (Eds.) *The Hippocampus: New Vistas*. A.R. Liss. New York.

Spence, J.T. & Maher, B.A. (1962) Handling and noxious stimulation of the albino rat: I. Effects on subsequent emotionality. *Journal of Comparative and Physiological Psychology, 55 (2)*, 247-251.

Squire, L. (1987) *Memory and brain*. Oxford University Press.

Stanton, M.E., Wallstrom, J. & Levine, S. (1987) Maternal contact inhibits pituitary-adrenal stress responses in preweanling rats. *Developmental Psychobiology, 20 (2),* 131-145.

Stanton, M.E. & Levine, S. (1989) Maternal modulation of infant glucocorticoid stress responses: role of age and maternal deprivation. *Psychobiology, 16 (3),* 37-42.

Stone, E.A., Egawa, M. & McEwen, B.S. (1988) Novel effects of chronic corticosterone treatment on escape behavior in rats. *Behavioral and Neural Biology, 50,* 120-125.

Sturrock, R.R., Smart, J.L. & Tricklebank, M.D. (1983) A quantitative neurohistological study of the long term effects in the rat brain of stimulation in infancy. *Journal of Anatomy, 136 (1),* 129-144.

Sutherland, R.J. & Dyck, R.H. (1984) Place navigation by rats in a swimming pool. *Canadian Journal of Psychology, 380 (2),* 322-347.

Sutherland, R.J. & Rudy, J.W. (1989) Configural association theory: the role of the hippocampal formation in learning, memory and amnesia. *Psychobiology, 39,* 45-59.

Takahashi, L.K., Kalin, N.H., Vanden Burgt, J.A. & Sherman, J.E. (1989) Corticotrophin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. *Behavioral Neuroscience, 103,* 648-654.

Teyler, T.J. & DiScenna, P. (1986) The hippocampal memory indexing theory. *Behavioral Neuroscience, 100,* 147-154.

VanHoesen, G.W., Human, B.T. & Damasio, A.R. (1991) Entorhinal cortex pathology in Alzheimer's disease. *Hippocampus, 1,* 1-8.

Vicedomini, J.P., Nonneman, A.J., DeKosky, S.T. & Scheff, S.W. (1986) Perinatal glucocorticoids disrupt learning: a sexually dimorphic response. *Physiology and Behavior, 36,* 145-149.

Weiss, J.M. & Gray, P. (1973) Hormones and avoidance behavior: a different approach points to a role for mineralocorticoids. *Progress in Brain Research, 39,* 471-480.

Wilson, M., Greer, S., Greer, M. & Roberts, L (1980) Hippocampal inhibition of pituitary-adrenocortical function in female rats. *Brain Research, 197,* 433-441.

Wilson, D.A. (1984) A comparison of the postnatal development of post-activation potentiation in the neocortex and dentate gyrus of the rat. *Developmental Brain Research, 16,* 61-68.

Wilson, D.A., Willner, J., Kurz, E.M. & Nadel, L. (1986) Early experience alters brain plasticity. *Behavioural Brain Research, 21,* 223-227.

Witter, M.P. (1986) A survey of the anatomy of the hippocampal formation, with emphasis on the septotemporal organization of its intrinsic and extrinsic connections. *Advances in Experimental Medical Biology, 23,* 67-82.

Wong, R. (1966) Infantile handling and performance in the T-maze. *Psychonomic Science, 5 (5),* 203-204.

Wong, R. & Jamison, J.L. (1968) Infantile handling and the facilitation of discrimination and reversal learning. *Quarterly Journal of Experimental Psychology, 20,* 197-199.

Wurtman, R.J., Pohorecky, L. & Baliga, B. (1972) Adrenocortical control of the biosynthesis of epinephrine and proteins in the adrenal medulla. *Pharmacological Review, 24,* 411-426.

Yongue, B.G. & Roy, E.J. (1985) Adrenalectomy fails to alter radial maze performance of rats at retention intervals of 24 hours or less. *Physiology and Behavior, 35,* 651-654.

Zola-Morgan, S. & Squire, L. (1986) Memory impairment in monkeys following lesions limited to the hippocampus. *Behavioral Neuroscience, 100,* 155-160.